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SEED AND SEEDLING INFECTION OF BARLEY BY RHYNCHOSPORIUM SECALIS[1]

W. P. Skoropad[2]

SUMMARY

The floral bracts, pericarp, and awns of barley seed are readily infected by *Rhynchosporium secalis* (Oud.) Davis, thus becoming sources of primary inoculum. Morphological features, including the remains of floral parts and barbs on the undersurface of the lemma and upper surface of the pericarp, are largely responsible for the location of scald lesions on the upper portion of the seed. Cool temperatures and adequate moisture favor barley germination as well as the production of conidia in the scald lesions. The coleoptile comes in contact with the inoculum as it grows past the underside of the lemma. The natural opening located near the tip of the coleoptile serves to trap the conidia and provides moisture for their germination. High soil temperatures inhibit the production of conidia and speed emergence of the coleoptile, thus preventing infection during germination of the seed.

Scald of barley, caused by *Rhynchosporium secalis*, is the major foliage disease of barley in many regions. Its importance as a limiting factor in barley production is growing with intensification of barley cropping and increasing adoption of shallow cultivation practices that leave straw at or near the surface. Like most leaf-infecting organisms, *R. secalis* overwinters predominantly as dormant mycelium on barley residues. However, the readiness with which the disease spreads to newly opened barley areas suggests that there is some means of long-range dissemination of the causal organism.

The possibility that the scald fungus is seed-borne has been investigated, but no general agreement has been reached and there has been no formal description of this phase of the disease. In 1929 Hauptfleisch (3), and more recently Pichler (4), suggested that seed-borne inoculum existed, but actual infection sites were not observed. Smith (7) referred briefly to scald symptoms on barley chaff, and in 1957 Reed (5) verified seed-transmission of the scald organism, mainly on the basis of field tests. Caldwell (1) reported negative results in attempts to demonstrate overwintering of scald on seed and dissemination of the organism by infested seed. Dillon Weston (2), in 1944, also concluded that the fungus is not seed-borne.

The present paper deals with the manner in which barley seed and seedlings are infected by the scald fungus. It reports the role of morphological features of the host, and the effect of environment on the parasite and host in initiating infection.

MATERIALS AND METHODS.—Infection of seed by the scald fungus was studied under field and greenhouse conditions. Seed infected under natural conditions was picked in barley fields with severe incidence of scald on the leaves. In the field, seed was infected artificially by spraying newly emerged spikes with a water suspension of conidia during cool, cloudy days. In the greenhouse, infection was good if a cool temperature and a high relative humidity were maintained for 2 days following inoculation. Selection of scalded seed from samples of threshed barley was facilitated by immersing the seed in water for 5 minutes, since wetting rendered the lesions considerably more conspicuous.

Infection of seed or seedling parts not showing characteristic scald symptoms was verified by floating the tissue on water at cool temperatures for 48 hours. The scald fungus sporulated abundantly under these conditions (6).

RESULTS.—*Seed infection under natural conditions.*—The manner in which conidia of *R. secalis* are spread in the field, and the conditions under which they germinate, determine to a large extent how barley seed is infected. Spore traps set out at various locations in or near barley fields showed that conidia were most abundant during rain storms. They usually appeared in clusters of 3–10, indicating that they were transported in droplets of water. This method of dissemination favored initiation of infection because a very high relative humidity or free water is essential for germination of conidia of *R. secalis* (1). During prolonged dry periods the number of conidia on the slides dropped sharply, and they usually appeared singly.

Infection of barley seed by *R. secalis* occurred most commonly at a time when the kernel had filled more than half the space enclosed by the floral bracts. It took place almost invariably on the inside of the lemma immediately below the base of the awn; the few other occurrences were on the inside of the palea (Fig. 1-A). In 6–10 days after infection, typical scald lesions, with a light center and a dark brown margin, appeared on the outer surface of the chaff (Fig. 1). The lesion on the lemma was frequently divided by the midrib, which produced a 2-eyed effect (Fig. 1-B).

The portion of the pericarp in contact with the infected regions of the lemma or palea was also infected but the lesion was less conspicuous than that on the floral bracts. The center was similar in color to the healthy portion of the pericarp, and the margin delimiting it was a light rather than a dark brown. In all instances mycelium was confined to the surface on which infection was initiated. It thus occurred on the undersurface of the lemma (Fig. 2) or palea, and on the outer surface of the pericarp (Fig. 3), and consisted of a tightly packed layer of short-celled hyphae.

Infection of seed occurring during the late-dough stage of barley did not always progress sufficiently to express scald symptoms on the outer surface of the

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[2] Plant Pathologist.

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Fig. 1–7. Fig. 1. Mature barley seed showing scald lesions **A)** on the tip of the palea, and **B)** on the lemma at the base of the awn. **Fig. 2.** Mycelium of *R. secalis* on the undersurface of the lemma. **Fig. 3.** Conidia and mycelium of *R. secalis* on the upper surface of the pericarp. **Fig. 4.** A natural opening below the tip of the coleoptile of barley. **Fig. 5.** An enlarged portion of the natural opening in the coleoptile, with conidia of *R. secalis* lodged in the depression. **Fig. 6.** A scald lesion on the tip of the coleoptile. **Fig. 7.** Types of emergence of the plumule from under the pericarp and lemma: **A)** emergence at embryo end prevents contact of coleoptile with inoculum in scald lesion; **B)** emergence at opposite end permits contact of coleoptile with inoculum in scald lesion.

lemma or palea. However, mycelium was present on the undersurfaces of these floral bracts as well as on the outer surface of the pericarp, and served as a hidden source of primary inoculum. Seed from samples of threshed barley taken at random from fields where scald had appeared on the leaves showed a disease incidence of 1–10%, averaging 2%. This did not include diseased seed on which symptoms were inconspicuous.

In susceptible varieties the awns were also readily infected. Conidia infected any portion of the awn, but did so most commonly at the base of the barbs. The glumes and the rachis were infected only rarely, even under ideal artificial conditions.

Morphological features of the developing seed and floral parts were largely responsible for the position of the scald lesions. Conidia were transported in tiny droplets of water and reached the distal end of the seed directly, or more commonly by way of the awns. Water droplets bearing the inoculum were trapped by the awns, and, after reaching sufficient volume, they rolled down between the tips of the slightly parted floral bracts. Further progress of the inoculum toward the embryo end of the seed was arrested by the cottony remains of the floral parts, the barbs and hairs on the inside of the lemma and on the outside of the pericarp, and enlarging kernel. An ideal moist chamber was established within the floral cavity, and conidia germinated readily. The dead anthers, pollen, and stylar tissues, besides acting as a sponge to retain moisture, also furnished nutritive material for the initial development of the scald fungus, which, once established as a saprophyte, penetrated the adjacent tissues.

Scald symptoms on the lemma were not observed at the embryo end of the seed under natural conditions. The barbs, hairs, and floral parts were apparently effective in arresting the water droplets at the distal end of the seed, where infection eventually occurred. Reduction of surface tension by adding 1 part of 0.02% Aerosol to 3 parts water allowed the water drops to get past these barriers. Forty per cent of the 100 florets that were inoculated with Aerosol in suspension resulted in infections at the embryo half of the seed, whereas no such infections occurred with inoculum lacking Aerosol. Thus, the surface tension of the water droplets appeared to be at least one important factor in determining the location of infection courts on the seed.

Most attempts to secure infection of the outer surfaces of the lemma or palea failed. Similarly, attempts to infect the undersides of these bracts by inoculation with viable, dry conidia resulted in very little success. However, inoculation with dry spores, followed by injection of a drop of water, gave good infection of the floral parts and the pericarp.

Seedling infection from infected seed.—Dormant mycelium on scald lesions sporulates abundantly within 48 hours under cool, moist conditions. The coleoptile comes into contact with this inoculum as it grows under the diseased pericarp and lemma. Actual contact with the fungus is made only when the plumule breaks through the pericarp at the lesioned end of the seed. The coleoptile develops a prominent scald lesion on its tip 4–6 days after emergence.

A natural opening in the coleoptile, located below its tip and on the side next to the lemma (Fig. 4), appears to influence the location of the lesion as well as the establishment of favorable moisture conditions for infection. This opening serves as a depression in which conidia lodge (Fig. 5) and are prevented from being brushed off as the coleoptile proceeds through the soil. Furthermore, this depression ordinarily contains free water of transpiration, thus acting as a moist chamber in which both germination of conidia and penetration of the host occur.

Disease development proceeds rapidly in the coleoptile, and a scald lesion is produced on its tip (Fig. 6). Conidia are produced abundantly in the drop of water that usually collects at this point, through which the first true leaf eventually emerges. The location of the scald lesion in this region facilitates the entrance of the inoculum into the funnel of the leaf whorl, and thus the embryonic tissues of the first true leaf are readily infected. In this manner, both the coleoptile and the leaf emerging from it become sites from which secondary inoculum is disseminated.

Effect of soil temperature and depth of seeding on seedling infection.—Since conidia of *R. secalis* are sensitive to heat (1), soil temperatures and the depth of seeding were considered as factors that may influence seedling infection from seed-borne sources of the fungus.

Fifty scald-lesioned seeds of the Moore variety were planted in pots of sterile soil at each of the combinations of 6 soil temperatures and 3 depths listed in Table 1. Optimum moisture was maintained in all pots. The percentages of infected seedlings emerging from such treatments are given in Table 1.

Depth of seeding appeared to have no bearing on per cent of infected seedlings. Although ineffective in controlling scald in the greenhouse, depth of seeding may have some practical value in the field to the extent that temperatures vary with depth. Seedling infection was optimum at a soil temperature of 16°, decreased sharply at 20°C, and was almost completely absent at 22°.

Effect of type of seed germination on seedling infection.—Data presented in Table 1 show that, even at optimum temperatures, about 20% of seedlings from diseased seed escape infection. A good portion of these escapes may be explained on the basis of the manner in which the plumule emerges from the pericarp. The plumule normally develops under the peri-

TABLE 1.—*Effect of soil temperature and depth of seeding on the amount of seedling infection from seed-borne sources of R. secalis*

Depth of seeding (in.)	Per cent of seedlings infected at					
	10°C	14°C	16°C	18°C	20°C	22°C
0.5	68	64	76	56	24	6
1.0	54	60	82	72	18	2
2.0	48	48	86	68	34	6

carp and lemma, and emerges at the top of the seed (Fig. 7-B), but a certain percentage of the coleoptiles emerge by breaking through the pericarp and lemma at a point near the base of the kernels, immediately following initiation of growth (Fig. 7-A). With this latter type of emergence, the coleoptile makes no contact with the scald lesion, thus growing out of the soil without being inoculated. In the two barley varieties under test, Moore and Gateway, an average of 12% of infected seed germinated in a manner that allowed the seedlings to escape infection.

The above observations were confirmed in the following manner. Lesioned seeds of Moore and Gateway were germinated on blotting paper at 16°C and divided into 2 lots, one with coleoptiles growing throughout the length of the kernel under the pericarp, and the other with coleoptiles breaking through the pericarp and lemma near the embryo end of the seed. Twenty-five seeds of each variety were selected from each type of emergence. These 4 seed lots were planted separately and grown at 16°C, the optimum temperature for scald infection. None of the seedlings that emerged from the embryo half of the kernel were infected, whereas over 90% of those that grew past the lesions were infected in each variety under test.

Discussion.—Caldwell (1) showed that mycelium of the scald fungus is restricted to that side of the leaf on which infection initially developed. This is also true for the lemma, palea, or pericarp. Smith (7) first reported typical scald lesions on the seed chaff, and this observation has been confirmed. Reed (5) did not refer to typical scald lesions on the floral bracts, although he mentioned that penetration by the scald fungus is limited to the lemma and palea and that the fungus is never found on the seed coat. The present study shows that infection of the pericarp almost invariably accompanied infection of the floral bracts.

High soil temperatures operated on host and parasite, lowering the incidence of seedling infection from seed-borne inoculum. Temperatures between 22° and 27°C retard sporulation of *R. secalis*, but favor rapid growth of the plumule. The seedling thus grows past the scald lesion before conidia are produced. Cooler temperatures have the reverse effect, slowing down growth of the plumule and speeding up sporulation of the fungus. Thus, inoculum is available when the coleoptile comes into contact with the scald lesion.

Since *R. secalis* sporulates prolifically and seedling lesions appear early, even a small percentage of well-spaced loci of conidial production is a potential for a scald epiphytotic in the field.

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Edmonton, Alberta

LITERATURE CITED

1. Caldwell, R. M. 1937. Rhynchosporium scald of barley, rye, and other grasses. J. Agr. Research **55**: 175-198.
2. Dillon Weston, W. A. R. 1944. Looking back and looking forward. (A retrospect of cereal diseases in East Anglia in the past 21 years.) Ann. Appl. Biol. **31**: 366-370.
3. Hauptfleisch, K. 1929. Über den Einfluss von Saatbeizmitteln auf das Auftreten von Marssonina graminicola an der Gerste. Nachbl. deut. Pflanzenschutzdienst **9**: 27-28.
4. Pichler, F. 1954. Die wichtigsten Krankheiten der Gerste. Pflanzenarzt **7**: 3-4.
5. Reed, H. E. 1957. Studies on barley scald. Tenn. Univ. Agr. Expt. Sta. Research Bull. 268.
6. Skoropad, W. P. 1957. An improved method of inoculating barley leaves with Rhynchosporium secalis. Phytopathology **47**: 445.
7. Smith, N. J. G. 1937. Leaf-scald of barley in South Africa. S. African J. Sci. **34**: 286-290.

DISEASE CONTROL THROUGH INTIMATE MIXING OF MERCURIC OXIDE WITH SOIL[1]

John Grainger

SUMMARY

A new type of machine for controlling various soil-borne pathogens delivers about 13 cu. ft. per acre of dust containing 5 lb. of Hg equivalent as yellow oxide of mercury and incorporates it evenly with the top 9 in. of soil. Yields of potatoes on ground infested with root eelworm (*Heterodera rostochiensis*) were increased sufficiently for economic use. Eelworm populations were reduced an average of 79%; consecutive applications would, under certain conditions, permit economic yield almost equal to that of a healthy crop, and appreciable residual effects were found in the second, and sometimes the third, season after a single treatment applied to a fairly low eelworm infestation. Control was also good from treatment at half the above rate. Club-root (*Plasmodiophora brassicae*) of turnips was reduced about 96%, and yield increased by as much as 9 tons per acre. The seed-borne pathogen that causes blackleg and those that cause diseases of the tuber at lifting time were not controlled. On the other hand, Rhizoctonia and Colletotrichum root rots on the growing crop, Spongospora tuber scab, and the soil-borne blackleg pathogen were substantially reduced.

A new kind of field-scale machine (Fig. 1) for intimate incorporation of nonvolatile materials with the cultivated depth of soil has recently been made[2] and described (6). This soil disease-control unit opens a fresh line of approach in controlling various parasites inhabiting the soil mass, and the present paper describes results when it was used to incorporate 13 cu. ft. (about 12 bu.) of dust[3] per acre containing 5 lb. of Hg equivalent as yellow oxide of mercury (YOM). These amounts now seem rather high, and it should be possible to effect adequate control at about £10 ($28) per acre, thus providing the least expensive soil-mass treatment now known. It will be shown that applications were effective in controlling potato root eelworm, *Heterodera rostochiensis* Wollenweber, a bacterial disease, and certain fungal pathogens in the soil.

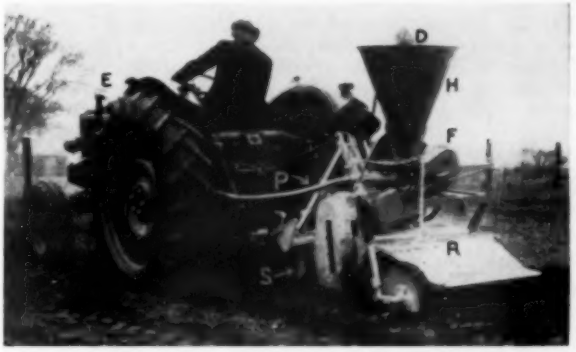

Fig. 1. The soil disease-control unit mounted on a tractor and in operation. **E,** exhaust control valve on the tractor. **P,** flexible pipe leading the diesel exhaust to pick up the dust from the hopper **(H). D,** dose-regulating mechanism. **F,** booster fan to assist dust delivery and mix it evenly with the exhaust stream. **R,** rotavator (under the cover). **S,** one of two side plates that limit the surface applications.

EQUIPMENT FOR CONTROLLING SOIL PATHOGENS.—Production of this machine was stimulated by the results of numerous pot experiments on controlling potato root eelworm. These demonstrated with various mercury compounds the efficiency of such small amounts as that mentioned above, provided they were mixed very intimately with the soil, originally by hand methods (5). Mercury compounds are already known as control agents for soil fungi, insects, and nematodes, but their efficiency is low unless intimately mixed. Johnson (7), for example, obtained only some 16–36% control of *H. rostochiensis* from about 200 lb./A. of mercuric chloride raked into the soil surface. The economics of obtaining greatly enhanced performance (up to about 94% control of the same disease) from treatment with as little as 5 lb. Hg per acre were tempting, so the purely scientific results of the pot experiments were converted into a practical field-scale proposition by work previously described in detail (6). The machine, which is a production prototype, achieves the necessary degree of intimate mixing by delivering dust at 2 levels—the surface and 7 in. deep—and then incorporating with a horizontal rotary cultivator (rotavator) that works to 9 in. deep. Surface deflectors are arranged to deliver dust evenly at ground level immediately in front of the rotavator, so that none has any chance to escape. Applications in depth are mechanized by blowing the dust into the spaces behind a series of duck-foot tines (Fig. 2). This arrangement gives the requisite distribution within ±20% of the required dose in all parts of the treated mass—a specification obtained by simulating good and bad methods of mixing, using radioactive iodine (I^{131}) in place of mercury for ease of determining distribution (5, 6).

METHODS.—The work with *H. rostochiensis* was necessarily done in a fenced, hygienically controlled area that, however, had fairly strong gradients of fertility and infestation. Particular experiments with fourfold replications were therefore arranged in different parts, hence the different untreated values of Tables 1 and 2. The positions of treatments in one

[1] Accepted for publication April 7, 1959.

[2] The production prototype was made to the desired specification (6) jointly by Rotary Hoes, Ltd., West Horndon, Essex; and E. Allman & Co., Ltd., Chichester. It was tested and modified mechanically at the Department of Plant Pathology, Auchincruive, Ayr, Scotland.

[3] Suitable dusts were compounded by F. W. Berk & Co., Ltd., London.

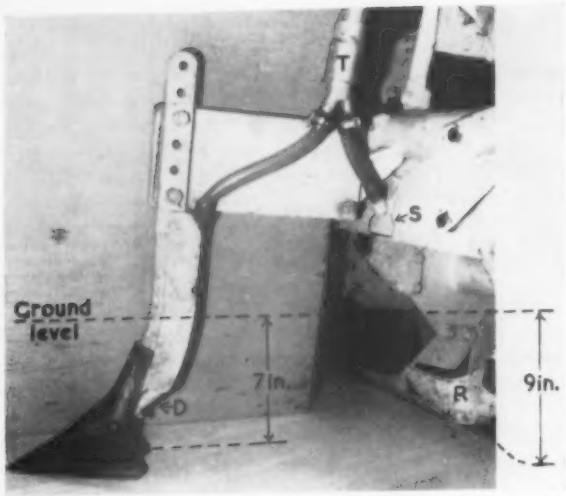

Fig. 2. Mechanization of the dust delivery at 2 levels. **T,** tube delivering dust from the booster fan (Fig. 1). **D,** delivery of dust into the space behind a duck-foot tine for application at 7-in. depth. **S,** a deflector for the surface application. **R,** rotavator tines working to a depth of 9 in.

year were marked, so that the action of consecutive annual treatments and residual effects could be assessed, though the area was not large enough for much replication on these aspects. Potatoes were grown in all 3 years 1956, 1957, and 1958. The eelworm population in untreated areas averaged about 0.2 cysts with contents per g of soil in 1956, 0.5 in 1957, and 1.3 in 1958, thus making it possible to obtain results from treatments applied to populations of different levels. Applications were all at the rate stated above unless otherwise mentioned, and yields were calculated to the equivalent rate per full acre from plots 1/170 acre in area.

The degree of eelworm infestation on potato roots was estimated as the number of cysts per inch of root by comparison with "standard length" diagrams (4). This is a relative method, but no other gives such a direct measure of the disease-producing ability of the eelworm population of the soil. Hyphae of *Rhizoctonia solani* Kuehn frequently accompany eelworm infestations, and the amount of infection was measured on roots of potato plants taken at random. The roots were washed, mounted in water between two microscope slides, and examined under a microscope with squared eyepiece micrometer. The length of the brown hyphae present could then be measured and related to the area of the root, a mechanical stage being used to select a new sample view for each determination. Infections of *Colletotrichum atramentarium* (Berk. & Br.) Taub. were found on potato roots in one experiment, and were estimated by a similar method, where, however, the number of sclerotia per hand-lens field (1.6 cm^2) was counted. Amounts of club-root (*Plasmodiophora brassicae* Woron.) on turnips were assessed by assigning individual plants into one of the 11 disease

TABLE 1.—*Control by YOM dust of root eelworm on Kerr's Pink potatoes (averages of all experiments, 1956–58)*

	Treated		Untreated		Effects of treatment[a]	
	Yield[b]	Cysts[c]	Yield[b]	Cysts[c]	% yield increase	% decrease in eelworm attack
Single treatments						
1. 1956, full rate	–[d]	0.08	–[d]	0.3	67*	74**
2. 1957, full rate	8.8	0.7	6.5	2.0	34*	66*
3. 1958, full rate	4.6	0.3	3.2	4.3	48	94*
4. 1958, full rate	6.4	0.3	4.8	3.1	33	90*
5. 1958, full rate	8.6	0.2	5.6	2.3	52*	93**
6. 1958, half rate	11.2	0.2	7.7	1.2	46*	87
Additional control:						
7. 1957, filler only	5.7	2.5	6.0	2.5	(0)	(0)
Consecutive treatments (unreplicated)						
8. Treated 1956 & 1957; result in 1957	10.1	0.7	6.3	3.0	61	78
9. Treated 1957 & 1958; result in 1958	9.5	0.2	4.8	3.1	98	93
10. Treated 1956, 1957, & 1958; result in 1958	11.8	0.2	4.8	3.1	147	94
Residual effects (unreplicated) Treated 1956; untreated 1957 & 1958[e]						
11a. Result in 1957	11.7	0.3	7.8	2.1	51	85
11b. Result in 1958	7.3	1.8	5.6	2.3	30	22
Treated 1957; Untreated 1958[e]						
12. Result in 1958	6.4	0.6	4.8	3.1	34	81

[a] Percentage effects of treatment and estimates of significance were calculated on the original data, before expression as the equivalent in tons per acre, and correcting to 1 decimal place. *Significant at the 5% level. **Significant at the 1% level.

[b] Tons of tubers per acre.

[c] Number of cysts per inch of root.

[d] Percentage increase in yield and decrease in eelworm attack from treatment could be estimated only on a crop planted in June. The actual yields of such a late crop bear no useful relation to the rest of the table, and are therefore omitted.

[e] Potatoes were grown in all years.

TABLE 2.—*Control by YOM dust of root eelworm on Epicure potatoes (averages of all experiments, 1958)*

	Time of lifting	Treated		Untreated		Effects of treatment[a]	
		Yield[b]	Cysts[c]	Yield[b]	Cysts[c]	% yield increase	% decrease in eelworm attack
Single treatments							
1a. Full rate	July 23	3.8	3.5	3.0	9.2	25	62*
1b. Full rate	Aug. 22	7.1	1.3	5.6	8.0	26**	84*
2a. Half rate	July 23	3.8	4.3	3.0	9.2	26	53*
2b. Half rate	Aug. 22	8.7	0.9	6.8	6.0	27	86
Consecutive treatments							
3. Treated 1957[d]; Treated 1958	July 23	4.4	3.2	3.0	10.3	48	69*
Residual effects							
4. Treated 1957[d]; Untreated 1958	July 23	3.0	7.6	3.0	10.3	2	27

[a]Percentage effects of treatment and estimates of significance were calculated on the original data, before expression as the equivalent in tons per acre and correcting to 1 decimal place. *Significant at the 5% level. **Significant at the 1% level.
[b]Tons of tubers per acre.
[c]Number of cysts per inch of root.
[d]Treatment was given to ground planted with Kerr's Pink in 1957.

categories (0–10) in steps of 10% of disease, by "standard area" diagrams (3). The number of plants in each category was then multiplied by the number of the category, and the products were added and expressed as a percentage of the total number of plants multiplied by 10. Similar disease rating methods were used for corky scab (*Spongospora subterranea* (Wallr.) Lagh.) and *Rhizoctonia* on stored tubers.

RESULTS.—*Potato root eelworm.*—Tables 1 and 2 show that, when YOM treatment was applied to ground infested with root eelworm, there were increases in potato yield and decreases in eelworm attack in all experiments, on 2 varieties, and over 3 years. In spite of the known variability in fertility and infestation of the only ground available, there was sufficient mathematical significance in the main results (rows 1–6 of Table 1, and 1 and 2 of Table 2) to warrant their acceptance. Row 7 of Table 1 gives results when the dust filler without mercury was used for treatment; its lack of both increase in yield and decrease in eelworm attack shows that the positive results given in the rest of Tables 1 and 2 must be due to the mercury. In general, the lower the eelworm population available to attack the roots, the greater was the yield increase, which is in line with a general interpretation of eelworm population values in relation to control (in preparation for publication). None of the populations treated was so high that no increase in yield was obtained.

Treatment at half the standard rate of dust and mercury mentioned above was almost as good as at the standard rate (*cf* the percentage results of rows 5 and 6, Table 1, and rows 1 and 2, Table 2). The standard rate might well be rather high for the most economic treatment. Treatments in consecutive years demonstrate the possibility of obtaining yields at almost the "healthy" level (row 10, Table 1), even though the results were not from replicated plots. Rows with consecutive treatments did, indeed, stand out, by their greater vigor of growth, from rows treated only in the current year (Fig. 3). The residual effects of one treatment are by no means negligible, being measurable up to the third year after treatment (row 11, Table 1). This is not likely to be a residual action of the mercury, but arises from reduction of the eelworm population to a very low level by the first treatment. The higher the population to which the first treatment was given, the less the residual effects could be expected to be, and some evidence of this is given by row 4, Table 2.

Values for cysts per inch of untreated root are at a lower level for the maincrop variety Kerr's Pink (Table 1) than for early Epicure (Table 2). This is because the maincrop estimations at lifting were of a later hatch of yellow eelworm cysts than those on the early crop. Sufficient earlier liftings of Kerr's Pink were made to show that the populations were similar on both varieties during July and August. Cysts of the first hatch on Kerr's Pink could, moreover, still be seen by detailed examination at lifting in September. They were, however, very dark in color, and so were not easily seen in normal field examination. Percentage diminutions by treatment of the later hatch on Kerr's Pink (Table 1) were somewhat greater than those of the main hatch on Epicure (Table 2).

It was noticed that potato root systems were larger on treated plants than on untreated ones, thus raising the question whether the diminutions shown in Tables 1 and 2 might not in fact be the distribution of equal numbers of cysts over systems of different sizes. Roots were therefore carefully dug and infestation measured, then washed, surface dried, and weighed. An aliquot weighed part was then measured, and an approximate length of the whole system calculated by proportion. Such a method can only specify the size of a major part of the root system, but multiplication of its length by the number of cysts per inch (an average value for the whole root mass) provides an approximation of the number of cysts formed per plant. This number was still lower on treated plants: 9000, against 22,500 untreated, for the main hatch on Epicure; and 1000, against 4500 untreated, for the later hatch on Kerr's

Pink. There had therefore been a real reduction of the attacking eelworm population as a result of treatment.

The total yield of each plot was, in 1957 and 1958, passed over screens (riddles), for separate measurements of the amounts of ware (edible standard), seed, and chat-sized (small) tubers. Screen or riddle sizes for ware and seed overlap; ware consists of all tubers large enough not to pass a 1¾-in. mesh; seed tubers lie between the 1¼-in. and the 2¼-in. meshes, and chats pass through the 1¼-in. mesh. Amounts of ware and seed therefore add up to more than the total yield, but each represents the effective yield from the point of view of the merchant of edible ware and of seed, respectively. The extensive data on tuber size are condensed in Table 3, which gives the averages of all experiments on both varieties in 1957 and 1958. Increases in total yield and amounts of seed and chats are still mathematically significant. There

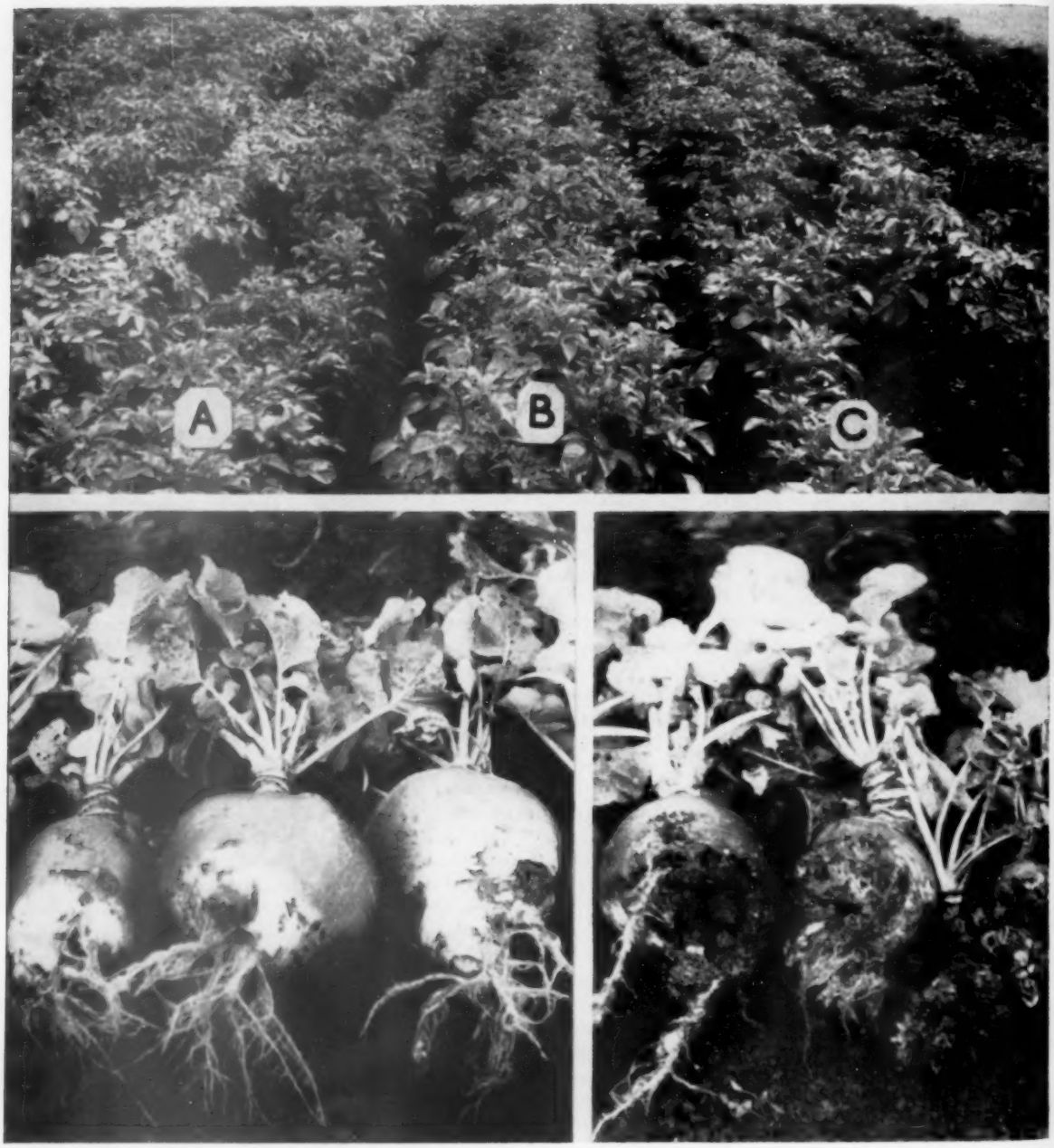

Fig. 3. Above: Three rows of Kerr's Pink potatoes, 1958. **A** and **C** were treated in 1958 only (included in row 4, Table 1). **B**, treated in 1957 and 1958, shows greater vigor of growth (included in row 9, Table 1). **Below**: Swedes, variety Best of All, from treated, left, and untreated plots, right (see Table 5); photographed at normal lifting in November, when the club-root galls had begun to decay.

TABLE 3.—*Effects of YOM treatment on potato tuber size (grand averages of all treated and control plots in 1957 and 1958)*

		Tuber grades and riddle (screen) sizes			
		Total	Ware (>1¾ in.)	Seed (1¼–2¼ in.)	Chats (<1¼ in.)
1. Yield (tons/A.)	Treated	7.75	3.84	5.69	1.09
	Untreated	4.96	2.29	3.49	0.76
	Increase	2.79*	1.55	2.20*	0.33*
2. Increase %		56	68	63	44
3. % of total yield	Treated	–	49	73	14
	Untreated	–	46	70	15
	Increase	–	56	79	12

*Significant at the 5% level.

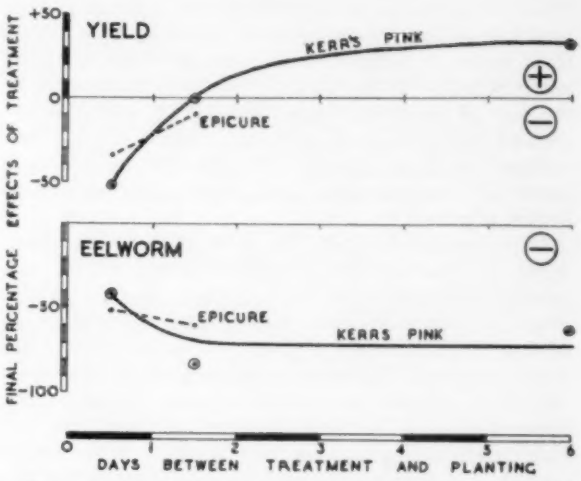

Fig. 4. The relation between time of treatment and yield increase; experiments in 1957.

are higher percentage increases of ware and seed than of chats over the untreated yield (row 2, Table 3), and, to put it another way, the increase also contains higher percentages of ware and seed and a lower percentage of chats than did the untreated crop (row 3, Table 3). Treatment had therefore enhanced the most commercially desirable tuber sizes of the total yield.

Treatment is expected to cost something like £10 ($28) per acre for materials, and the cost of application is largely offset by savings on costs of normal cultivation, since the equipment provides the necessary tilth straight from plowed ground. Ordish (8) has published results suggesting that a ratio of cost to benefit of 1:3½ is necessary to tempt farmers to use crop protection measures on a medium-value crop like potatoes. At the very moderate price of £16 ($44.80) per ton for maincrop produce, an increase of about 2¼ tons would be required to give this ratio value. Such increases, or larger ones, are shown in rows 2, 5, 6, and 11a of Table 1, and, allowing for the increased costs of successive treatments, by rows 9 and 10 also. Early Epicures generally sell for £40 ($112) to £50 ($140) per ton, and something like a ¾-ton increase would meet the ratio of cost to benefit. Such increases are shown by the early liftings 1a and 2a in Table 2, though they would in practice be grown even earlier in the year. Later-lifted Epicures in west Scotland are usually for seed, and about a 1½-ton increase would be necessary (1b and 2b, Table 2) to provide the suitable ratio. Treatment is therefore likely to be economically tempting to growers.

A suitable interval must separate treatment and planting, as was found from results in 1957 (not included in Tables 1 or 2 but shown in Fig. 4). Planting 12 hours after treatment was followed by a large decrease in yield; planting after 36 hours gave no in-

TABLE 4.—*Effects of YOM treatment on potato diseases*

	Treated	Untreated	% effects of treatment*
Diseases on the growing crop			
Rhizoctonia root rot of potato, mm of brown hyphae per cm² root			
Epicure, July 25, 1958	1.4	3.2	–55
Kerr's Pink, pot expt. Aug. 1, 1958	0.9	3.5	–73
Kerr's Pink, Sep. 6, 1958	0.2	1.9	–91
Colletotrichum root rot of potato, sclerotia per cm²			
Kerr's Pink, Aug. 1958	19.7	34.7	–43
Bacterial black-leg, % of infected plants			
Kerr's Pink, soil-borne infection, 1958[b]	0.2	1.2	–83
Epicure, seed-borne infection, 1958[b]	6.9	6.8	0
Phytophthora-blight-infected tubers, % by weight at lifting			
Late; 125 days, treatment to lifting	4.3	6.0	–28
Late; 116 days, treatment to lifting	6.4	8.1	–21
Normal; 142 days, treatment to lifting	6.1	5.5	+10
Tuber diseases after storing (Jan. 1959)			
Rhizoctonia black scurf, % by area	0.2	0.2	–14
Fusarium dry rot, % of infected tubers	1.7	2.0	–15
Spongospora scab, % by area	0.1	1.4	–92

*These were calculated on the original data; results in the other columns are corrected to 1 decimal place.
[b]See text.

crease or decrease, but planting 6 days after treatment gave a substantial increase in yield. This early phytotoxic action is believed to be due to yellow oxide of mercury before it is reduced to the elemental form in the soil as demonstrated by Booer (1), who also explained the action of mercury compounds in controlling soil-borne disease (2). Results in Tables 1 and 2 are all from treatments given more than 6 days before planting.

Other potato diseases.—Table 4 shows results from YOM on several other potato diseases. They are mostly not suitable for mathematical analysis of significance, and only those with a high percentage of disease reduction are worthy of note. These are Rhizoctonia and Colletotrichum rots of potato roots (which frequently accompany eelworm attack), soil-borne bacterial blackleg, *Erwinia atrosepticum* (v. Hall) Jennison (but not this disease when tuber-borne), and Spongospora tuber scab. The virtual absence of tuber-borne blackleg on Kerr's Pink (Table 4) was checked by growing the stock on clean land, when the number of infected plants was minute (about 0.001%). The experimental ground, on the other hand, had been infected by growing Epicures with tuber-borne disease in the previous year. The relatively poor control of the other diseases listed in Table 4 is considered further in the discussion.

Club-root of turnips and swedes.—Very good disease control (95-98%) was obtained on ground with a persistent, heavy infection with *Plasmodiophora brassicae* (Fig. 3 and Table 5). The variety Best of All is completely susceptible to club-root and is a swede—a turnip of hard flesh and about 11% dry matter, and capable of relatively long storage. Bruce is a fairly resistant softer turnip with about 9% of dry matter, and not so good for storage; Wilhelmsburger is a moderately resistant swede. All showed increases in yield from treatment, though Bruce was the highest, with a 9-ton increase on this land of rather low fertility. It is hoped to experiment further in 1959, and with lower rates of treatment.

Discussion.—Evidence has been presented for the control of potato root eelworm, four soil-borne fungus diseases, and the soil phase of a bacterial pathogen. Do the results allow us to go further and form an idea as to how long the mercury treatment is active? A working hypothesis has indeed emerged—that the effects of treatment persist well into the cycle of growth, but that the effect is almost exhausted by the time the normal growth of potato and turnip is over. In fact, it appears that the effectiveness of treatment increases until about the end of August. Percentage decreases in eelworm attack were quite modest, 53-62%, for July liftings of Epicure (rows 1a and 2a, Table 2); they increased to 84-86% for August liftings (rows 1b and 2b, Table 2), and were highest of all, 87–94%, for September-lifted Kerr's Pink (rows 3–6, Table 1). The percentage control of Rhizoctonia root rot of potato also increased between July 25 and September 6 (Table 4).

The lack of effect at the end of growth is suggested by the slight control of tuber diseases at lifting or after storage (Table 4). Indeed, the only disease on the tubers found to be controlled by a significant amount was Spongospora scab, infection by which begins to appear at an early stage of tuber formation, when it would be under the influence of the mercury treatment. *Rhizoctonia* was diminished by a considerable amount on the growing roots, but only to a negligible amount on the tubers. Fusarium dry rot infection appears at lifting time and was not significantly diminished by treatment. Phytophthora blight appears on the tubers only toward the end of growth; it was not controlled on a normal crop (there was actually a slight increase of 10%), but on 2 late-treated crops in 1957 and 1958 there were slight decreases in the amounts of tuber blight. It is probable that gangrene, caused by *Phoma foveata* Foister, is also in the same category, but more work is necessary. There are, then, already several results in favor of the working hypothesis, to be checked by further work.

It has been shown that the new soil disease-control unit can bring efficient economic disease control in the field, using mercury dust at a rate lower than that of many less efficient partial treatments already in horticultural use. There are, however, numerous other materials that might control disease more efficiently if they were intimately incorporated, and there are other

Table 5.—*Control by YOM dust of club-root on turnips and swedes (averages of all experiments, 1958)*

Variety	Time of lifting	Treated		Untreated		Effect of treatment[a]	
		Yield[b]	Club-root[c]	Yield[b]	Club-root[c]	% yield increase	% decrease in club-root
Best of All							
	Aug. 30[d]	7.3	0.6	4.4	41.5	66	98
	Nov. 26	20.3	0.6	12.6	36.9	61*	98*
Bruce							
	Aug. 30[d]	10.3	1.0	7.3	17.8	41	95
	Nov. 26	26.8	0.2	17.8	11.8	50**	98*
Wilhelmsburger	Nov. 26	20.1	0.5	18.3	11.0	10	96**

[a] Percentage effects of treatment and estimates of significance were calculated on the original data, before expression as the equivalent in tons per acre and correcting to 1 decimal place. *Significant at the 5% level. **Significant at the 1% level.
[b] Tons of turnips per acre.
[c] Percentage; see text.
[d] Unreplicated.

soil-borne diseases for which an economic control is urgently needed.

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LITERATURE CITED

1. BOOER, J. R. 1944. The behaviour of mercury compounds in soil. Ann. Appl. Biol. **31**: 340-359.
2. BOOER, J. R. 1951. The action of mercury as a soil fungicide. Ann. Appl. Biol. **38**: 334-347.
3. GRAINGER, J. 1950. Crops and diseases. West Scot. Agr. Coll. Research Bull. **9**: 1-51.
4. GRAINGER, J. 1951. The golden eelworm. West Scot. Agr. Coll. Research Bull. **10**: 1-72.
5. GRAINGER, J. 1956. Progress in soil mixing for nematode control. Nematologica **1**: 31-46.
6. GRAINGER, J. 1958. A new soil disease control unit. West Scot. Agr. Coll. Research Bull. **25**: 1-46.
7. JOHNSON, L. R. 1936. Trials of mercuric chloride for the prevention of potato sickness. Ann. Appl. Biol. **23**: 153-164.
8. ORDISH, G. 1955. The economics of crop protection. Chem. & Ind. London. 1955: 24-30.

THE RELATION OF ENVIRONMENT TO THE RINGSPOT (MYCOSPHAERELLA BRASSICICOLA) DISEASE OF CRUCIFERS [1]

Merritt R. Nelson and Glenn S. Pound [2]

SUMMARY

Using both mycelial and ascosporic inocula of *Mycosphaerella brassicicola*, relatively low temperature and high humidity were required for infection and sporulation on cabbage. Infection occurred over the range 12–28°C, but levels of infection were much lower at 12° and 24° than at 16° and 20°, and only a trace occurred at 28°. Lesions developed most rapidly at 20°, but the greatest number were produced at 16°. Infection did not occur unless inoculated plants were kept in a saturated or near-saturated atmosphere for about a week. Perithecia bearing ascospores were produced consistently in a temperature range of 8–20°. Fruiting bodies were also produced at 4°, 24°, and 28°, but these did not produce mature spores. The optimum temperature for ascospore formation was 16–20°. Spores matured slightly earlier at 20° than at 16°, but the ultimate production was apparently equal at both temperatures. Moisture also was required for sporulation. Mature perithecia did not develop on lesions until they were exposed to a saturated or near-saturated atmosphere for at least 4 days. If exposed for shorter periods, spores did not mature. Thus, these studies explain the restriction of the disease to certain coastal areas where low temperatures and long periods of high humidity prevail. *M. brassicicola* was found to grow best on cultural media at 20°, decreasing in order at 16°, 24°, 12°, 28°, 8°, and 4°. Growth was best on plant-extract media. No appreciable growth occurred on a completely synthetic medium unless thiamin was added, in which case growth was as good as on any medium.

The ringspot disease of crucifers, caused by *Mycosphaerella brassicicola* (Fr.) Lindau, is limited strictly to certain coastal areas of the world and in these areas it may be very destructive (7). It has been considered to occur only under certain conditions of atmospheric moisture and temperature, but the specific limits of these conditions have never been worked out. This paper presents detailed information on the specific environmental factors important in initiation and development of the disease. Data are presented to show temperature and moisture requirements for both infection and inoculum development.

Environmental requirements most likely play the principal role in restricting the disease, but other factors may also be of significance. Several workers have suggested that ringspot is seedborne (6, 10, 12). Others point out that not only is evidence supporting this inadequate, but evidence to the contrary is available (8). If ringspot is effectively seedborne, then restriction by environment must be very important. On the other hand, if the disease is not transmitted by seed, this fact would also restrict the spread of the disease. Thus it is possible that lack of effective seed transmission and strict requirements for environmental factors both function in limiting this disease to definite areas.

MATERIALS AND METHODS.—A single-ascospore isolate of *M. brassicicola* obtained from naturally infected cabbage provided by Dr. R. D. Raabe, of the University of California, was used in all experiments. The cabbage variety Jersey Queen was used exclusively for greenhouse infection studies. In any one experiment, all plants were the same age and had been grown under the same conditions.

Temperature studies were carried out in greenhouses with controlled temperatures of 12, 16, 20, 24, and 28°C.[3] In each house there was an inoculation chamber at the same temperature, so that plants were at a constant temperature throughout an experiment.

Plants infected in the greenhouse and containing

[1] Accepted for publication April 14, 1959.
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[3] All temperature values used are in degrees centigrade unless otherwise stated.

relatively few lesions were used for studying perithecial development. These lesions, previous to exposure to a saturated atmosphere, showed no fruit body development. Two methods were used to study the effect of temperature on perithecial formation and ascospore maturation. In one, lesions were left intact on the plants, which were transferred from an open greenhouse bench to inoculation chambers that were in the various temperature houses. After 7 days' exposure to a saturated atmosphere at the various temperatures, the plants were again removed to open greenhouse benches at the same temperatures. Beginning at 6 days after exposure to moisture, pieces of lesions were removed daily from the plants and inverted over a Petri plate containing water agar. When ascospores matured they were forcibly discharged and could be easily seen on the agar surface with a microscope. Examination was also made by crushing lesion pieces that had perithecia. In this way the development of immature asci could be observed. In the second method, excised lesions from plants not exposed to moisture were inverted over a Petri plate by placing them on a water-agar block stuck to the lid of the dish. Daily inspection of the agar area directly beneath the lesion revealed when the first ascospores were discharged, and hence the time required for ascospore maturation at the different temperatures. Laboratory incubators, used in the work with excised lesions, provided temperatures of 4, 8, 12, 16, 20, 24, and 28°.

Refrigerated inoculation chambers were used in which the temperature was set at 16° for studies on the effect of moisture on infection. Moisture requirements for infection were determined by leaving inoculated plants in a saturated atmosphere for various periods. To study the effect of daily alternation of wet and dry conditions, plants were removed from the inoculation chambers and kept on a greenhouse bench for specified periods. Time of penetration was established by washing inoculated leaves free of inoculum with a soft cloth and distilled water at various intervals after inoculation.

Studies on the requirements for atmospheric moisture for perithecial formation and ascospore maturation were carried out in both greenhouse and laboratory. In the greenhouse, the time required to initiate perithecial formation under moist conditions was determined by leaving plants with lesions in a saturated atmosphere for different periods. Lesions were checked for perithecial and ascospore development as already described. In the laboratory, relative humidity was controlled in small chambers by use of the glycerol solutions described by Cruickshank and Mueller (2). For 100% RH (relative humidity), distilled water was used; for 98% RH, a solution of 8.5 parts glycerol; for 96% RH, 16.5 parts; and for 95% RH, 19.5 parts of glycerol. Thin Petri plates (9 cm) were used as humidity chambers. The lesions were placed on circular cover slips mounted on glass rings in the humidity chambers, with the surface of the lesion as near the top of the dish as possible, and incubated at 20° (±0.1°C). Although the RH values given for these glycerol solutions were at 25°, any error encountered because of the temperature differential was felt to be insignificant, since under the conditions of the experiments, there should have been little difference between the RH values at 20° and 25°.

Studies on the effect of temperature on growth of *M. brassicicola* in PDA (potato-dextrose agar) cultures were carried out in laboratory incubators with temperatures at intervals of 4° in the range 4–32°. The carbohydrate levels tested were 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%. Growth factor requirements were determined with techniques established by Beadle and Tatum (1). In growth factor work all test tubes and equipment were acid washed. Bacto-agar and reagent grade dextrose and chemicals were used.

TECHNIQUE FOR PRODUCING INFECTION.—To obtain specific information on environmental requirements by greenhouse studies, it was essential to develop an inoculation technique that gives adequate and consistent infection. Since *M. brassicicola* does not fruit readily or abundantly on artificial substrate, and since there was not a ready source of ascospores from nature, the inoculation technique must employ mycelium. This fungus grows extremely slowly. A colony originating from a single spore reaches a diameter of 20 mm only after 7 weeks at optimum temperature, and on a suitable medium. It was, therefore, necessary to find a way of producing quantities of mycelium in relatively short periods.

Several cultural methods were found to produce mycelium. The most important factor was the introduction of quantities of mycelium to start the culture. Several ml of a mycelial suspension of a pure culture were poured, under aseptic conditions, into the medium used for shake cultures, and over the surface of agar slants. The liquid cultures, after 3 weeks on a shaker, contained good sized mycelial balls.

Petri-plate cultures were established in 2 ways: 1) by pouring the mycelial suspension into the molten agar before the plates were poured, in this way impregnating the entire medium with mycelium; and 2) by pouring the mycelial suspension onto the agar and spreading it evenly over the surface. Development of mycelium was fair in each case, but not as abundant or as quickly developed as on large-volume slants. Six-ounce pharmaceutical bottles, each containing 45 ml PDA (1% dextrose), were used to make slants. When grown on these slants, in contrast to Petri plates and low-volume slants, the fungus not only grew faster and to a greater diameter, but grew deeply into the agar as well. On low-volume slants or plates it grew for a time and then slowed, in some cases definitely stopping. Some nutrient or essential growth factor was apparently being used up because of the large number of growth centers.

In all cases the mycelium was harvested after 2–4 weeks of growth and homogenized with water in a Waring Blendor. Mycelial balls from shake cultures were first washed free of the culture medium in which they were grown. Mycelium from agar-slant cultures was at first merely scraped from the surface of the cultures, but it was later found that grinding up a third of an inch of the top of the culture with water

Fig. 1. Lesions produced on cabbage leaf with mycelial inoculum.

and using it as inoculum greatly increased the amount of infection. This increased infection could have been due to the fact that the agar provided a sticker to keep the slow-growing fungus longer in contact with the leaf or that some enzyme or toxic substance produced by the fungus and held in the agar enhanced penetration. In liquid cultures such a substance could have been lost by washing.

Weimer (12), attempting to infect plants with mycelium, had little success. In the present study, various methods were used in holding the inoculum in contact with the leaf. Little success resulted from merely placing pieces of mycelium from agar cultures on the leaf surface or using leaf-sized squares of cheesecloth that were soaked in a mycelial suspension and placed on the infection court. Injuries and abrasives were also used in attempts to increase infection, but these, too, were without success. Adding soybean extract or sucrose to the mycelial suspension failed to add to either the rate or the amount of infection.[4]

It soon became evident that inoculum grown on agar cultures was superior to liquid-grown inoculum. When agar was ground up with the mycelium, as already described, and used as inoculum, levels of infection were consistently high (Fig. 1). These results were first obtained with cheesecloth squares, but when inoculations were attempted without them, results were

[4] Acknowledgment is made to Dr. J. C. Horton for making available the results of inoculation experiments performed by him.

TABLE 1.—*Number of lesions on 4 leaves 30 days after Mycosphaerella brassicicola inoculum applied to upper and lower leaf surfaces*

	Upper surface only	Lower surface only	Both surfaces
Plant 1	21	155	216
Plant 2	32	207	270
Plant 3	31	94	213

similar. For example, in one experiment the respective lesion totals on 16 leaves at 23, 27, and 31 days after inoculation were 32, 150, and 200 with cheesecloth, and 36, 142, and 202 without. This agar-mycelial inoculum is a viscous liquid, and when applied to leaves, it forms a thin film over the leaf surface, even when dry. The fungus is thus kept in intimate contact with the leaf surface for long periods.

When leaves were inoculated on one surface only, those inoculated on the underside had 3–6 times as many lesions as those inoculated only on the upper surface. Leaves inoculated on both surfaces generally showed more infection than leaves with either surface inoculated singly (Table 1). Since the numbers of

Fig. 2. Symptoms produced on cabbage seed pods and stalks with mycelial inoculum.

TABLE 2.—*Number of lesions on 30 leaves (10 plants with 3 leaves each) of cabbage inoculated with agar-mycelium inoculum and incubated at various temperatures for various periods*

Days after inoculation	12°	16°	20°	24°	28°
16	0	213	423	74	0
20	10	1048	1177	135	3
24	59	1371	1290	190	11
28	129	1650	1408	234	37

TABLE 3.—*Number of lesions on 6 leaves (2 plants of 3 leaves each) of cabbage inoculated with ascospores and incubated at various temperatures for various periods*

Days after inoculation	16°	20°	24°	28°
16	5	11	0	0
20	23	31	3	0
24	49	47	8	0
28	54	49	14	0

stomata on the upper and lower leaf surfaces do not differ greatly (ratio about 5:7), the main reason for this large difference is most likely due to the washing effect of water collecting on the upper leaf surfaces during the long periods in the moist chambers.

Several crucifers were successfully infected with the inoculation technique described. These included cabbage, broccoli, cauliflower, Chinese cabbage, and cabbage seed plants. The leaves of many crucifers have a waxy coating that prevents wetting the surface. Such wax was easily removed before inoculation by rubbing the leaf surface with a piece of wet cheesecloth.

Cabbage seed plants were heavily infected in the greenhouse with the mycelial inoculation technique. In extreme cases seed pods became almost completely covered with lesions. The seed stalks also developed heavy infection, and some branches of the main seed stalk were completely girdled by lesions (Fig. 2).

EFFECT OF ENVIRONMENT ON INITIATION AND DEVELOPMENT OF DISEASE.—*The effect of temperature on infection.*—Inoculations were made one day after the plants were placed in the controlled-temperature greenhouses, and after 10 days in the inoculation chamber the plants were removed to an open greenhouse bench.

The effect of temperature was very pronounced on both the rate and amount of infection. Lesions appeared on plants at all temperatures, but some differences in symptom type were observed among the different temperatures. Disease development was greatest at 16° and 20°. Symptoms appeared earlier and developed more rapidly at 20°, but more lesions were usually produced at 16° (Tables 2, 3). Infection was much lower at 12° and 24°, and very few lesions formed at 28°. At 28° the lesions failed to enlarge and did not produce any fruiting bodies. Lesion enlargement at 24° was also less than at the lower temperatures but greater than at 28°. At 12°, and occasionally at 16°, chlorotic borders were found around the lesions. One experiment was conducted with ascosporic inoculum produced by mature lesions. Although the inoculum dose was low, the temperature response was the same (Table 3).

Alternating-temperature experiments were conducted by placing inoculated plants in the 28° temperature house for daily exposures of 4, 8, and 16 hours (the plants were otherwise at 16°). The appearance of symptoms was only slightly retarded when plants were exposed at 28° for only 4 hours per day. Exposures of 8 hours and 16 hours at 28°, however, markedly reduced the amount of infection (Table 4).

Effect of temperature on perithecial production and ascospore maturation.—After one week in the greenhouse inoculation chamber, a majority of the fruiting bodies at 12°, 16°, and 20° had reached a size within the range for mature perithecia. At 24° the largest bodies fell somewhat short of being full size, and those at 28° were considerably smaller. The fruiting bodies formed at 28° remained more or less spherical—rather than pyriform, as do mature perithecia—and had a ragged surface. At 12°, 16°, and 20°, perithecial development proceeded normally. Ascospores were first formed at 20°. At this temperature, immature asci appeared in 5 days after exposure to moisture, and ascospores were found 7 days after exposure to moisture, although this time varied somewhat with lesions of different ages. Ascospores at 16° and 12° generally appeared 1 day later and 3 days later, respectively, than at 20°. No spores were found on material from 24°, but immature asci were occasionally found. Nothing resembling asci or ascospores was ever found at 28°.

Studies with excised leaf lesions revealed that perithecia and ascospores were formed at relatively low temperatures. Spores were first found at 20° on the water agar plates after 7 days. Spores were usually found at 16° at the same time, but where large numbers were found at 20° at this time only a few were found at 16°. At 12°, 2–3 weeks were required for mature ascospore discharge. Asci were found in crushed material from 8° after 3 weeks, but spores did not appear until almost 4 weeks after initial exposure to moisture. No spores were found at 24° or higher.

Effect of atmospheric moisture on infection.—It was early observed that periods of several days in inoculation chambers were required for infection. It was thought that the pathogen may require relatively long periods of contact with the host before penetration could occur. If this were true, moisture would be necessary to sustain the fungus until it had become

TABLE 4.—*Number lesions on 16 leaves (4 plants of 4 leaves each) incubated daily at 28C[a] for various periods*

Days after inoculation	0 hours	4 hours	8 hours	16 hours	24 hours
21	320	217	58	15	0
27	411	329	105	47	6
33	452	416	155	71	10

[a] Plants were kept at 16° when not at 28°C.

TABLE 5.—*Time required in saturated atmosphere for infection by Mycosphaerella brassicicola to occur*

Treatment	Average number lesions on leaves of plants removed from moist chamber after stated number of days[a]					
	3	4	5	6	7	8
Washed leaves[b]	1	.5	1	6.3	12	24
Unwashed leaves[c]	0	1.5	5.7	13	11.5	21

[a] Notes were taken 22 days after inoculation.
[b] Inoculum removed from leaves by rubbing; plants left in moist chamber 7 days.
[c] Plants with these leaves unwashed were removed from moist chamber at the time indicated.

established within the suscept. To test this hypothesis, inoculated leaves were washed at various intervals after inoculation so as to remove inoculum. Moisture requirements were correlated with time of penetration by removing plants with unwashed leaves from the inoculation chambers at the same time that leaves on other plants were washed free of inoculum. The results indicated that penetration was slow, taking up to a week to be extensive. Moisture experiments correlated very well, indicating that the infection process is very slow (Table 5).

The time required for penetration, as determined by lesion development, was affected by the time of year. In winter experiments, leaves washed free of inoculum before 7–8 days showed no significant infection, whereas in the spring, penetration occurred about 5 days after inoculation.

Although long periods were required in the inoculation chamber for infection to occur, it was shown by alternating wet and dry conditions daily that the moisture need not be continuous. When inoculated plants were removed from the inoculation chambers for periods of 4, 8, or 16 hours daily, infection was obtained in all cases, although the rate and amount decreased as the dry period increased (Table 6).

The significance of these experiments is difficult to evaluate. The inoculum used, which forms an agar film on the leaf, may hold enough moisture so that a drying period does not actually dry out the imbedded mycelium as fast as it would seem. Since this experiment was not done with spores, the effect of drying under conditions in the field could not be determined.

Effect of atmospheric moisture on perithecial formation and ascospore maturation.—Ringspot lesions do not form fruiting bodies on leaves that have not been exposed to moisture conditions near saturation. After 24 hours in an inoculation chamber at 20°, however, small black spherical bodies can be observed (Fig. 3-C). If the plant is removed at this time, no mature bodies of any kind are developed. If the plants are allowed to remain in a saturated atmosphere, constantly or intermittently, for 4–6 days, mature perithecia will be formed. The first asci and ascospores are found at 7 days or longer after first exposure to moisture, depending on the temperature. Plants removed from moist chambers in less than 4 days usually do not form mature perithecia. The reason for the moisture requirement seems clear. Fruiting structures are formed on the lesions, which are necrotic tissue; hence, without moisture from a source other than the plant itself, fruiting does not occur. Areas near the edge of lesions and near large leaf veins are frequently observed to support earlier and more rapid perithecial development. The plant may thus be able to supply some moisture to places such as these, but, on the whole, an atmospheric source of moisture seems essential.

The relative humidities at which ascospore formation occurs were determined by the use of Petri-plate humidity chambers. Spherical black bodies were formed in 1–2 days at 100% RH, 3–4 days at 98% RH, and 6–7 days at 96% RH. When examined at 3 weeks, ascospores were found at 100 and 98% RH, but not at lower humidities. Large numbers were formed at 100% RH, but relatively few at 98% RH.

IN VITRO STUDIES OF M. BRASSICICOLA.—*Effect of temperature on growth.*—All cultures were started from single spores derived from a single-ascospore isolate. Growth was maximum at 20°, and decreased at other temperatures, in the following order: 16°, 24°, 12°, 28°, 8°, and 4°. Growth was very slow at 4°, taking 6 months for a colony to reach 25 mm in diameter. At the other temperatures, however, growth was relatively rapid. A colony reached a diameter of 30 mm in 10 weeks at the optimum temperature (20°). No growth occurred at 32°, and several days of exposure to this temperature resulted in the death of the fungus.

Attempts to stimulate fruiting in culture.—Snyder (9) was able to produce both perithecia and the spermatialike bodies of *M. brassicicola* on corn-meal agar in culture. In the present study, many kinds of media were tested to see if any would stimulate fruiting. These included common vegetable extract agar media such as corn meal, oatmeal, cabbage-leaf extracts, pea, potato dextrose, soybean meal, and malt, as well as natural media consisting of plant parts sterilized with propylene oxide (11). Plants used to prepare natural media included cabbage leaves and stems, pea stems, wheat straw, barley straw, Brussels sprouts stems, sweet pea stems, and cauliflower stems. Cultures were maintained up to 12 months in incubators at various temperatures, and under conditions of natural light and temperature. Cultures on window sills in the winter were exposed to temperatures between 10° at night and 24° in the daytime. Light is apparently not required for fruiting, since perithecia formed abundantly on lesions in dark incubators. Old

TABLE 6.—*Number of lesions on 15 leaves (5 plants of 3 leaves each) removed from dry conditions to moist chamber for various hours each day*[a]

Days after inoculation	24 hours	20 hours	16 hours	8 hours	0 hours
17	135	62	20	4	0
21	755	169	172	36	0
28	2271[b]	1742	1064	287	0

[a] Constant temperature at 20°C, in both moist and dry environments.
[b] Lesions were unusually numerous in this experiment, conditions for infection apparently being ideal.

cultures were sometimes replenished with water agar or other agars to see if this might stimulate fruiting. In no case did fruiting occur.

Effect on growth of different carbohydrate levels.— It has been observed that there are often differences between cultures of *M. brassicicola* on different media. On media rich in carbohydrates, a fluffy white mycelium often forms on the surface of the colony, whereas this characteristic is much less common on less rich media. To determine the effect of carbohydrate levels on the cultural characters, potato agar was used with dextrose added in the following amounts: 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0%. Differences in amount of growth were not great at these different levels of

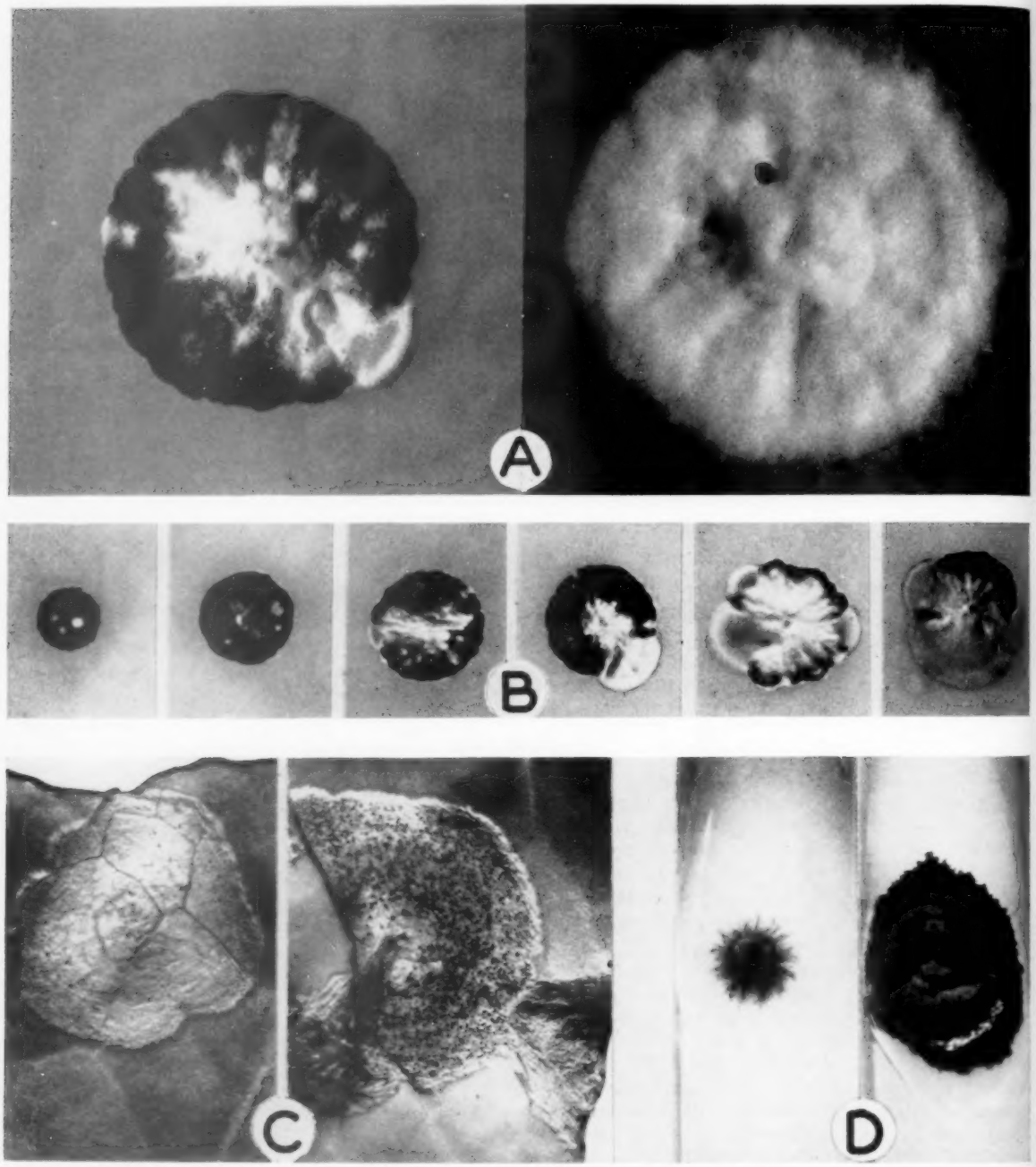

Fig. 3. A) Two single-ascospore cultural types obtained from greenhouse plants. **B)** Culture type produced on medium containing 0, 0.5, 1.5, 2.0, 2.5, and 3.0% dextrose, left to right, respectively. **C)** Effect of atmospheric moisture on perithecial formation: left, lesion on plant from open greenhouse bench; right, comparable lesion from plant kept in saturated atmosphere for 24 hours. **D)** Growth type and rate on basal salts media deficient in thiamin (left), and containing thiamin (right).

carbohydrate, but slight increases in growth and in the white mycelial character occurred with increase in the level of carbohydrate (Fig. 3-B). This growth character may be controlled to a certain extent by adjusting the level of sugar in the medium. The explanation for the occurrence of these white cultures is not that simple, however. When a large number of single-spore cultures were made with ascospores from greenhouse material, not only did this white culture show up regularly, but all gradations were found between it and the all-black culture type. The cultures that were completely white grew faster than the more common black cultures (Fig. 3-A). This white character would also appear after several mycelial transfers. No change in pathogenicity could be demonstrated when this occurred. The white mycelium is strictly limited to the surface of the colony, with the characteristic black mycelium always found underneath.

Extent and type of growth on various media.—*M. brassicicola* grew well on a number of different media, but best on media made from plant extracts. Linear growth was best on corn-meal agar and on agar made from a hot-water extract of fresh cabbage leaves. Growth on these media was quite filamentous, while most richer media supported a more compact, sclerotial-like growth. On a weight basis, most growth occurred on the richer media, such as potato-dextrose, soybean, and oatmeal agars. On a basal-salts medium, growth was filamentous, but stopped in a relatively short time. The colony formed was not the deep black usually formed in culture, but a lighter brown. While the margin of a colony on adequate media was smooth, the margin of these colonies was very irregular. When very minute amounts of yeast extract were added to basal salts media, growth was greatly stimulated. The stimulation from such a small amount of material strongly suggested that a growth factor may be required by *M. brassicicola*.

Growth factor requirements.—Media were prepared with a "shotgun" mixture of vitamins, casein hydrolysate, and purines and pyrimidines. When these media were inoculated with single spores, growth was normal only on the one having the shotgun vitamin mixture. Next, thiamin, riboflavin, pyridoxine, calcium pantothenate, *p*-amino-benzoic acid, choline, *i*-inositol, biotin, and nicotinic acid were put into media individually to see which vitamin was required. The fungus grew well only on the medium containing thiamin (Fig. 3-D). It is thus interpreted that *M. brassicicola* has a naturally occurring requirement for thiamin.

Weimer (12) noted that on synthetic media a feathery type of growth occurred. He also noted that a certain proportion of ringspot lesions on infected plants had the same irregular shape as cultures on synthetic media and were much smaller than the normal lesions. In the present study it was observed that when a relatively small number of lesions form on a leaf they develop normally to the maximum size. If a large number of lesions form, however, very few ever develop to any extent and the majority have decidedly irregular margins. Thus it would seem that lack of lesion enlargement and the reason for the irregularly shaped lesions are due to an inadequate supply of the vitamin in the leaf of the suscept. No fruiting bodies of any kind form on such lesions even when placed under conditions of heavy moisture.

DISCUSSION.—Data obtained in greenhouse studies show clearly that the most favorable temperature for disease initiation and development is 16–20°. This range is also optimum for ascospore production and maturation.

Alternating temperature experiments revealed that plants inoculated and placed at 28° for as long as 16 hours per day (otherwise at 16°) showed significant infection whereas plants continuously at 28° showed very little infection. Thus it seems possible that infection could occur under fairly warm conditions providing the nights were cool. If the disease is seedborne, lesions could develop in inland areas. The failure to get spore production at 24° and 28°, however, would not permit perpetuation of the disease.

Atmospheric moisture is also essential for infection and ascospore production by *M. brassicicola*. Without moisture for 5–7 days after inoculation plants did not form lesions. This moisture need not be continuous. Plants in inoculation chambers only one third of each day were infected, although much less than plants continuously in a saturated atmosphere.

These studies explain the marked restriction of environment on ringspot distribution. In the Puget Sound area, the coastal area of Oregon, and the San Francisco Bay area, where the disease occurs, temperatures are persistently low. Night temperatures during the growing season range from 45–60°F. Day temperatures occasionally reach 75–80°, but often range in the 60's to 70's. The monthly mean temperatures (°F) based on records from the 30-year period 1921–50 at Seattle, Portland and San Francisco are as follows:

	Seattle	Portland	San Francisco
January	41	39	50
February	43	44	53
March	47	48	55
April	52	54	56
May	57	59	57
June	62	64	59
July	66	68	59
August	65	68	59
September	61	64	62
October	54	56	61
November	47	47	57
December	43	42	52

Coupled with the persistently moderate temperature is abundant moisture. The immediate coastal areas are often enshrouded with fog and plants are in a very moist atmosphere for considerable periods. Thus the temperature and moist conditions are ideal both for infection and inoculum build-up. As one moves inland to a point where this combination of temperature and moisture does not prevail, one ceases to encounter the disease.

Field material obtained through the courtesy of

R. D. Raabe was examined carefully on several occasions during these investigations. Perithecia were often found but by far the majority of the fruiting structures were the spermogonialike bodies. This may have been due to the time of year or the conditions at the time of collection. In the greenhouse, however, careful examination of sporulating lesions revealed only perithecia. Spermogonia were sought under all conditions of temperature and moisture in these investigations, but without success except in only one instance, when a few spermatialike bodies were found. Higgins (3, 4, 5) has presented evidence that shows fairly conclusively, for several members of the genus *Mycosphaerella*, that the sexual apparatus for fertilization consists of spermatia and archegonia. Snyder (9) has suggested that the pycnidial-like bodies associated with ringspot, long thought to be a *Phyllosticta* imperfect stage of the fungus, are in fact spermogonia and function as such. The evidence he cited to support this is sound. In view of the greenhouse studies, however, where only perithecia were formed, it would appear that some modification of Snyder's suggestion must be made. It may very well be that in nature these small bodies function as spermatia, but because of the ease with which perithecia are formed in the greenhouse without the presence of spermogonia, it would appear that another mechanism, at least as efficient as the sexual process described by Higgins, exists. Histological investigations on greenhouse material are now in progress and may reveal the answer to this question.

Snyder showed that *M. brassicicola* is homothallic. That work is confirmed in this present investigation. Abundant perithecia were formed on lesions that developed when inoculum derived from a single ascosporic isolate of *M. brassicicola* was used.

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LITERATURE CITED

1. Beadle, G. W., and E. L. Tatum. 1941. Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. U.S. **27**: 499-506.
2. Cruickshank, I. A. M., and K. O. Mueller. 1957. Water-relations and sporulation of Peronospora tabacina Adam. Nature **180**: 44-45.
3. Higgins, B. B. 1920. Morphology and life history of some ascomycetes with special reference to the presence and function of spermatia. I. Am. J. Botany **7**: 435-444.
4. Higgins, B. B. 1929. Morphology and life history of some ascomycetes with special reference to the presence and function of spermatia. II. Am. J. Botany **16**: 287-296.
5. Higgins, B. B. 1936. Morphology and life history of some ascomycetes with special reference to the presence and function of spermatia. III. Am. J. Botany **23**: 598-602.
6. Huber, G. A., and C. J. Gould. 1949. Cabbage seed treatment. Phytopathology **39**: 869-875.
7. Pound, G. S. 1946. Diseases of cabbage plants grown for seed in Western Washington. State Coll. Wash. Agr. Expt. Sta. Bull. 475. 27 pages.
8. Pound, G. S., Fen-Ching Cheo, O. H. Calvert, and R. D. Raabe. 1951. Extent of transmission of certain cabbage pathogens by seed grown in Western Washington. Phytopathology **41**: 820-828.
9. Snyder, W. C. 1946. Spermogonia versus pycnidia in Mycosphaerella brassicicola. Phytopathology **36**: 481-484.
10. Snyder, W. C., and K. F. Baker. 1943. Diseases of seed cabbage in Calif. Plant Disease Reptr. **27**: 394-398.
11. Snyder, W. C., and H. N. Hansen. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology **37**: 420-421.
12. Weimer, J. L. 1926. Ringspot of crucifers caused by Mycosphaerella brassicicola (Fr.) Lindau. J. Agr. Research **32**: 97-132.

THE INFLUENCE OF TEMPERATURE ON ROOT ROTS OF POINSETTIA CAUSED BY THIELAVIOPSIS BASICOLA, RHIZOCTONIA SOLANI, AND PYTHIUM ULTIMUM[1]

D. F. Bateman and A. W. Dimock[2]

SUMMARY

The root rots of poinsettia caused by *Thielaviopsis basicola*, *Rhizoctonia solani*, and *Pythium ultimum*, show 3 different types of temperature response. The optimum temperature for growth of *T. basicola* on potato-dextrose agar was 24°C when based on increase in colony diameter and 21°C when based on increase in dry weight. For *R. solani* and *P. ultimum*, the respective optima were 30° and 27°C, regardless of the basis of determination. With all 3 fungi, the optimum temperature range was much greater when growth was determined by increase in colony diameter than when determined by increase in dry weight; this was particularly true for *Rhizoctonia* and *Pythium*, which show rapid hyphal elongation at temperatures considerably below optimum.

Root development of healthy poinsettias was most prolific at soil temperatures near 26°C. No growth occurred at 13°, and growth was considerably reduced at soil temperatures as high as 30°.

The effect of soil temperature on development of each of the 3 poinsettia root rots was studied with the aid of thermostatically controlled temperature tanks. Thielaviopsis root rot developed best at 17°C but was severe from 13° through 26°. At 30°, the highest temperature employed, little more than a trace of root rot developed. Pythium root rot also developed best at 17° but caused no serious damage at 26° or above. *Rhizoctonia*, which causes both a root rot and a stem rot of poinsettia, did not cause damage at temperatures below 17°. Disease severity increased as temperature was increased from 13° through 30°.

The root rots of poinsettia (*Euphorbia pulcherrima* Wild.) caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr., *Rhizoctonia solani* Kühn, and *Pythium* spp. have for many years been major obstacles to the profitable production of poinsettias. For the most part our knowledge concerning the effects of environment upon these diseases is inadequate, although such factors as soil temperature, moisture, pH, and saprophytic microflora undoubtedly influence their occurrence and development markedly. Elucidation of the environment-disease relationships may point the way to better control of these maladies, since the commercial crop is grown under greenhouse conditions, where the findings of environmental studies may, within certain limits, be applied. Reported herein is a study of the influence of one environmental factor—temperature—upon growth of the pathogens, growth of the host, and development of the diseases.

LITERATURE REVIEW.—Considerable research has been reported on the effects of temperature upon the growth of *T. basicola*, *R. solani*, and *P. ultimum*, and upon the development of diseases initiated by these organisms on several agronomic hosts. Very little such work, however, has been done with ornamental plants, where the results of such studies could most readily be applied.

Thielaviopsis.—Jones, Johnson, and Dickson (13) reported the optimum temperature for an isolate of *T. basicola* from tobacco to be between 28° and 30°C. Rawlings (24), working with isolates of *T. basicola* from tobacco, cotton, and *Primula obconica*, reported better growth at 20° than at 10° or 30°, and better growth at 10° than at 30°. Barnett, Lilly, and Waters (4) reported that *T. basicola* grows better at 25° than at 20°, 30°, or 31.5°. Lucas (20), working with isolates from Kentucky, Wisconsin, Virginia, North Carolina, and California, all of which were pathogenic on tobacco, found that all showed maximum growth between 22° and 28°.

Temperature has been considered one of the most important environmental factors influencing the development of black root rot of tobacco (6). The optimum temperature for development of this disease is between 17° and 23°C; a sharp decrease in severity can be noted at 26°; and very little root rot develops at 29–30°. If temperatures are maintained as high as 32°, the disease does not develop (13). Thielaviopsis root rot of cotton has also been reported to be more severe at low temperatures than at high temperatures (5), and the same has been reported for Thielaviopsis root rot of poinsettia (7, 14, 28).

Pythium.—The optimum temperature for growth of *P. ultimum* and closely related species from chrysanthemum, sugar cane, sweet potato, and poinsettia lies between 28° and 32°C (9, 10, 31, 32). In most cases where optimum temperature has been reported for disease development initiated by Pythium on its various hosts, disease has been most severe at temperatures unfavorable for growth of the host, with little relation to the temperatures favoring fungus growth (3, 10, 12). This being the case, one would predict that Pythium root rot of poinsettias would be most severe at low temperatures, since it has been shown that growth of the host is adversely affected by low temperatures (23).

Rhizoctonia.—The group of organisms identified by the binomial *Rhizoctonia solani* is a heterogeneous one. Isolates of this fungus have been divided into at least 3 distinct groups based on culture morphology

[1] Accepted for publication April 15, 1959.

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(11). Also, the various optimum temperatures reported for growth of *R. solani* and for disease development on its various hosts further indicate its heterogeneity. Richards (25) felt that the temperature requirements for pathogenic action of *R. solani* on its various hosts is a fixed heritable character of the fungus, influenced little by the temperature requirements of the hosts, since he found that the isolates he was working with inflicted the most severe damage upon pea and potato (cool-temperature crops) and bean and cotton (warm-temperature crops) at 18°C. The origin of a given isolate of *R. solani* appears to dictate to a large degree the optimum temperature for its growth as well as the temperature at which it will inflict the most damage upon its hosts. A brief survey of the literature indicates that most isolates of *R. solani* fall into 2 major groups as far as optimum temperature for growth is concerned. Most isolates obtained from hosts favored by high temperatures grow best in culture at temperatures near 30°C and inflict greatest damage near this same temperature (1, 18, 29, 33). In contrast, it appears that isolates obtained from hosts favored by low temperatures grow best in culture at or below 25° and inflict the most severe damage upon their hosts at temperatures near or below 20° (16, 25, 26). There are, of course, some Rhizoctonia isolates that do not conform entirely to the above generalization (15, 22).

MATERIALS AND METHODS.—*Thielaviopsis basicola* was isolated from poinsettia stock plants at Ithaca in May, 1957, with the carrot-disk technique described by Yarwood (34). *Pythium ultimum* and *Rhizoctonia solani* were isolated from diseased poinsettia plants in North Carolina in September, 1957. All 3 isolates proved to be exceedingly virulent and were used throughout this investigation.

Laboratory studies.—Experiments were designed to determine the optimum temperature for growth of each of the above pathogens in pure culture. The fungi were grown in 90-mm Petri plates containing about 10 cc of potato-dextrose agar (PDA). In each experiment, all plates were poured from the same batch of medium to ensure uniformity. They were seeded centrally with 1-mm^3 blocks of inoculum taken from the margins of actively growing colonies. The temperature range employed was from 9° through 36°C at 3° intervals. Temperatures were maintained within ±1°C over the course of the experiments. Six replicate plates of the *Rhizoctonia* and *Pythium* isolates and 4 of the *Thielaviopsis* isolates were incubated at each temperature during each experiment. Each fungus was tested at least twice at each temperature, excluding preliminary tests.

During preliminary tests, measurements of colony diameter were made for each fungus, at each temperature, at 12-hour intervals during the entire incubation period. This proved unnecessary, and later measurements were taken only at the end of the incubation period. This period varied with each fungus, since it was determined by the time required for the given organism to cover the agar surface at its "optimum" temperature. Determination of mycelial dry weight has been considered the more precise method of measuring fungus growth (19), hence this method was employed. After colony diameters were measured, the colonies and supporting agar were removed from the Petri plates and placed in storage dishes containing 200 ml of distilled water. These were then placed in an Arnold steam oven for 20 minutes. This freed the mycelium from the agar; the colonies were then removed and washed. Dry weights were determined after the mycelial mats had been dried for at least 24 hours at temperatures between 95° and 100°C.

Greenhouse studies.—The effect of temperature on the development of each of the poinsettia root rots, as well as on the root growth of healthy plants, was determined in thermostatically controlled soil-temperature tanks (8) in the greenhouse.

Cuttings (var. Barbara Ecke Supreme) were selected for uniformity from vigorously growing stock plants. They were rooted in a specially constructed rooting tray provided with adequate drainage and equipped with an electric heating element that maintained a temperature of about 26°C in the vermiculite rooting medium at all times. The cuttings were rooted under intermittent mist. Under these conditions, rooting was excellent in 15–20 days, even during the winter.

The potting soil was a mixture of ⅓ sand, ⅓ peat, and ⅓ field soil by volume. The mixture was about pH 7 after sterilization by autoclaving for 2 hours at 15 lb. pressure. The pH did not vary more than 0.3 unit between different batches but in each experiment a single batch of soil was used.

Five soil temperatures were used, ranging from 13° through 30°C at about 4° intervals. Temperatures were maintained within ±1°C. In the late summer and early fall, it was unnecessary to water the plants in the 13° tank, since condensation was considerable at this temperature, thus maintaining a high moisture level. At the other temperatures, plants were watered as needed in order to keep the moisture content at all temperatures comparable. The moisture content was about 28% on a dry-weight basis.

Inoculum was grown on PDA at room temperature (21–26°C). Ten-day-old cultures of *Pythium* and *Rhizoctonia*, and 4-day-old flood-plate cultures of *Thielaviopsis* were employed.[3] The sterilized soil was infested uniformly by mixing fragmented inoculum at the rate of 1 Petri-plate culture to 5 quarts of soil, this amount of soil being used for each 10 plants in a given experiment. Healthy uniformly rooted cuttings were potted in the infested soil mixture in steam-sterilized 4-in. clay pots and placed immediately in steam-sterilized 1 gal. glazed crocks in the temperature tanks. Each crock was equipped with a drainage well so that excess moisture could be removed if necessary (Fig. 1). Each tank contained 15 crocks. As controls, 5 plants were grown in uninfested soil in each tank during each experiment. The control plants also served to determine the effect of temperature upon the

[3] The solidified agar surfaces of prepared Petri plates were flooded with a pure spore suspension of the fungus and incubated for 4 days.

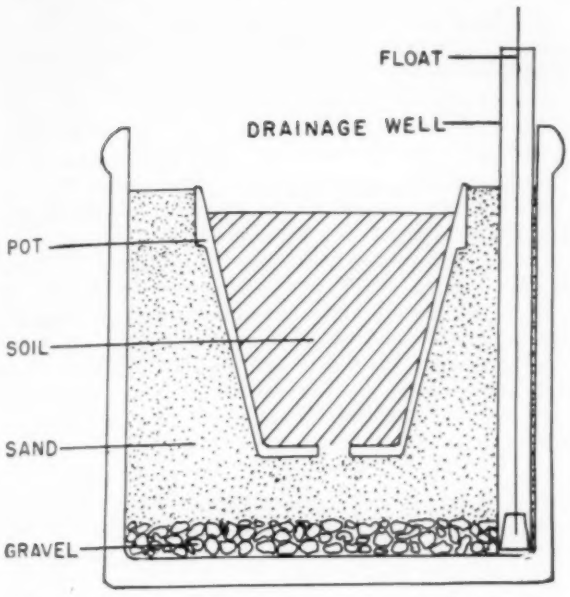

Fig. 1. Drawing showing the cross-sectional view of a 1-gal. crock and supplementary parts prepared for use in the constant soil-temperature tanks.

root growth of healthy plants.

Data in all cases were taken 2 weeks after the plants had been placed in the temperature tanks. Preliminary tests indicated that this was the best time to examine the roots if maximum differences were to be observed at the various temperatures under the conditions described above. The root systems were washed free of soil, care being taken to minimize breakage and loss of roots. They were then rated visually according to the following index: 0 = healthy root system, 1 = trace of root rot, 2 = about ¼ roots rotted, 3 = about ½ roots rotted, 4 = about ¾ roots rotted, and 5 = all roots rotted. An attempt was made to determine root rot severity by taking fresh root weights. This method, however, did not seem to give a valid indication of root rot severity, since it was almost impossible to remove all of the soil and debris from severely rotted root systems without losing portions of decayed roots. The problem was further complicated by the different amount of growth occurring in healthy plants at the various temperatures. For example, no root growth occurred at 13°C in either the infested or uninfested soil, and diseased and healthy roots consequently weighed about the same. Therefore, only the disease index values were used as a measure of disease severity. The root weights of healthy plants, on the other hand, were considered a valid indication of normal root growth.

RESULTS.—*Growth of the pathogens in culture.*—Results of growth-rate determinations are shown graphically in Fig. 2. *Pythium ultimum* grew best at 27°C, and its optimum temperature range was 24–33°C. Growth fell off very rapidly above 33°. Very little growth occurred at 9°, 12°, and 36°. *Rhizoctonia solani* grew best at 30°; no growth occurred at 9° and very little at 12°, 15°, and 36°. The optimum temperature range for this fungus was 27–31°. For *Thielaviopsis basicola,* the optimum temperature range was 15–27°; optimum growth was at 24°, on the basis of colony diameter, and at 21°C on the basis of mycelial weight. Considerable growth occurred at 9°, the lowest temperature in the series, but no growth occurred at or above 33°.

A comparison of the growth curves of the 3 fungi (Fig. 2) suggests several differences in the 2 methods of measuring fungus growth. Colony diameter reflects only radial extension or the ability of the organism to spread over the substrate, whereas dry mycelial weight represents total growth. With *Pythium* and *Rhizoctonia,* mycelial development was definitely sparser at low than at high temperatures. With *Thielaviopsis* this relationship was reversed. The optimum temperature range for growth of these organisms is greater when determined by colony diameter alone than when determined on a dry-weight basis. This was particularly true for *Pythium* and *Rhizoctonia,* since they

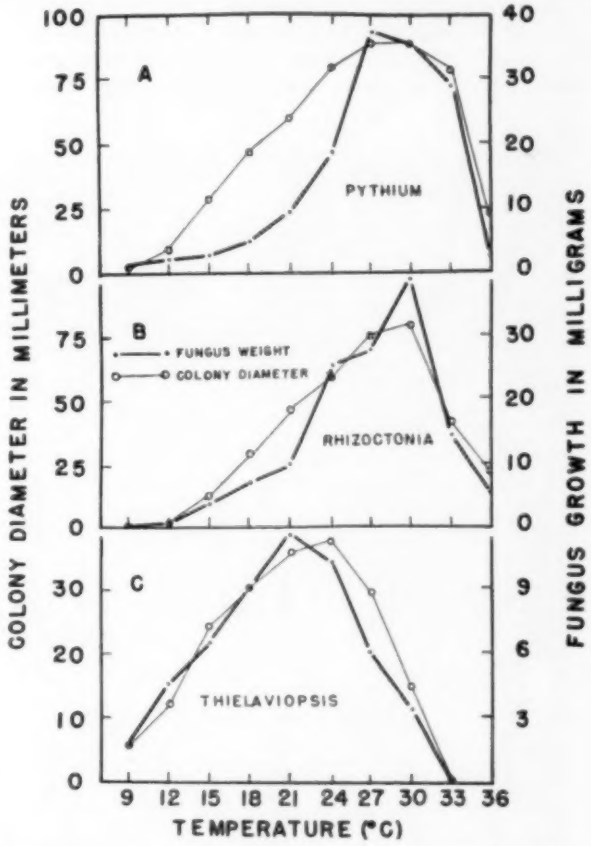

Fig. 2. Effect of temperature upon the growth of **A)** *Pythium ultimum;* **B)** *Rhizoctonia solani,* and **C)** *Thielaviopsis basicola* on potato-dextrose agar as determined by colony diameter and dry mycelial weight. These measurements represent the average of 2 experiments with 6 replicate colonies of *Pythium* and *Rhizoctonia* and 4 replicate colonies of *Thielaviopsis* at each indicated temperature. Both measurements were made from the same thalli in each case. The incubation periods were 41, 48, and 120 hours, respectively, for *Pythium, Rhizoctonia,* and *Thielaviopsis.*

spread rapidly over the substrate at temperatures considerably below optimum.

With both *Pythium* and *Rhizoctonia* the respective optimum temperatures for growth were the same regardless of the method used for determination. Leach (17), in contrast, on the basis of dry mycelial weight of the fungus grown on potato-dextrose liquid medium, reported a much lower optimum for *P. ultimum*, namely, 20°C, whereas his results based on colony diameter on PDA, however, were similar to those reported here. This suggests that there may be a considerable difference in the optimum temperature for growth, depending on whether the fungus is grown on liquid or solid media, regardless of which method of measuring growth is employed.

Temperature and root growth.—The effects of temperature upon the root growth of poinsettia were marked (Fig. 3-A). At 13°C, no growth occurred over the course of the relatively short-term experiments here employed. This is in agreement with the work of Post, Bing, and Horton (23), who found that poinsettias grow very little, if at all, at temperatures below 60°F (15.5°C). Root growth was considerable at 17°C, this growth being due chiefly to the initiation of new roots at the base of the plants, rather than to branching and elongation of existing roots. At 21°, root growth was extensive; the root system became elongated and highly branched, the branching being profuse near the base of the older roots. The same type of development occurred at 26°, the only differences being that growth was a little more massive than at 21°. At 30°, growth fell off considerably; the root systems were elongated but very little branched, and very few new roots were initiated.

Root rot symptoms.—*Thielaviopsis basicola* appears to be able to attack the root system at almost any point. In soil of high inoculum content, as used in these studies, a root system speckled by numerous light brown lesions may be considered characteristic of early infection. The lesions are not necessarily congregated near the points where new roots emerge from older roots, as has been described for tobacco (6). As the disease progresses the entire root system may turn brown and later become black because of formation by the fungus of characteristic black-walled chlamydospores. In the later stages of infection, longitudinal black cracks, aparently initiating at lenticels, appear on the lower portion of the stem, the black color again resulting from the presence of chlamydospores. The latter symptom is definitely characteristic

Fig. 3. Typical root systems of **A)** healthy, **B)** *Thielaviopsis*-infected, and **C)** *Rhizoctonia*-infected poinsettias after 14 days of incubation at the following constant soil-temperatures (left to right): 13°, 17°, 21°, 26°, and 30°C.

of *Thielaviopsis* infection and distinguishes it from Rhizoctonia and Pythium rots.

Pythium ultimum commonly initiates infection near the root tips, particularly in small roots. Once infection has occurred and conditions are favorable, the fungus gradually progresses up the root system. In cases where disease is mild, infection may be limited to the few roots originally infected, but these are usually completely destroyed. This is particularly true at high soil temperatures. Under conditions of

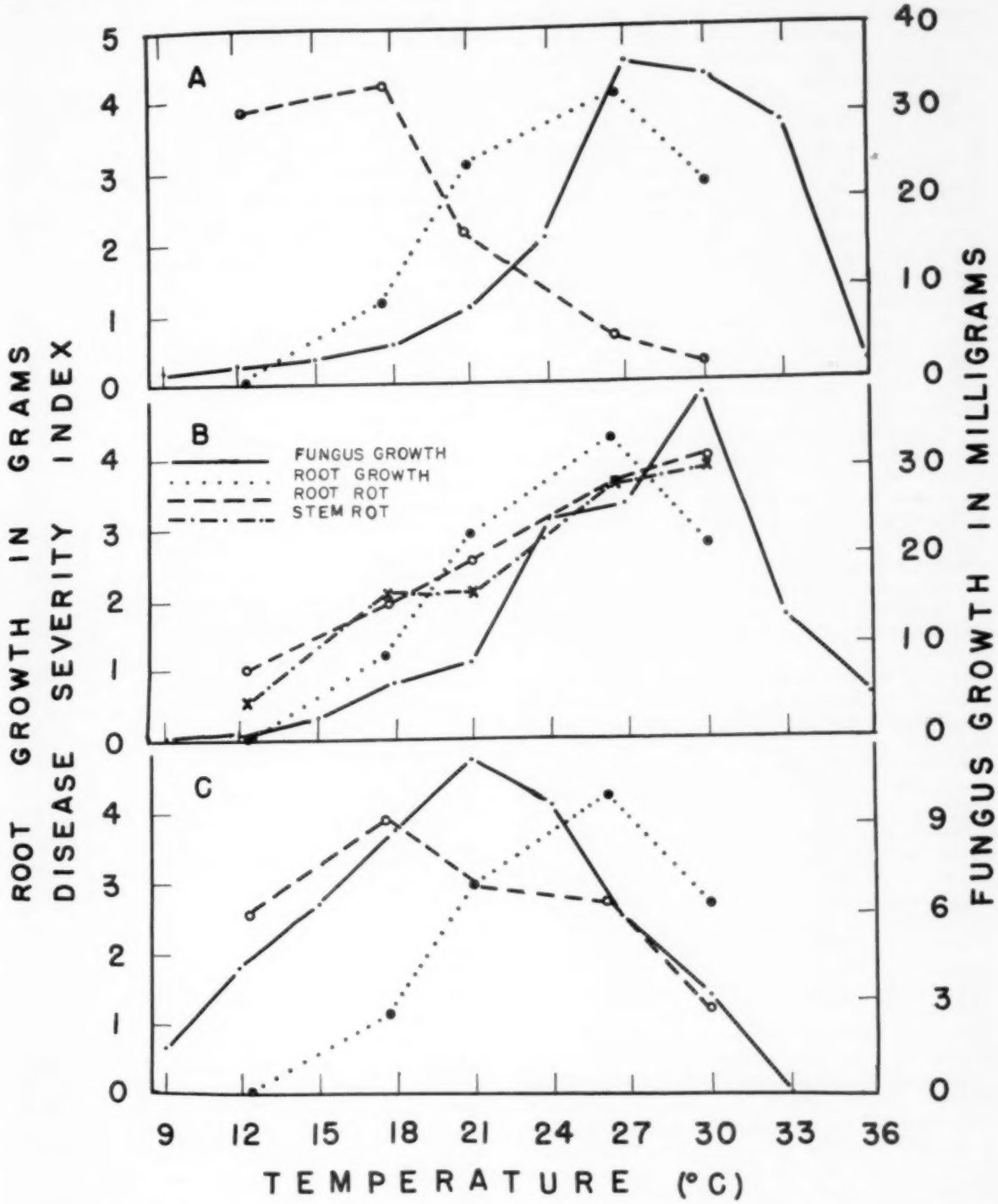

Fig. 4. The influence of temperature upon root growth of poinsettia, growth of the pathogen in culture, and disease severity. A) *Pythium ultimum*, B) *Rhizoctonia solani*, and C) *Thielaviopsis basicola*.

low soil temperature and high soil moisture, most of the roots become infected, and the entire root system is destroyed in time. Pythium root rot is characterized by a light brown color and definite water-soaked appearance. Tompkins and Middleton (31), working with several *Pythium* spp., including *P. ultimum*, observed a stem rot as well as a root rot caused by these organisms. Stem lesions initiated by *Pythium* on rooted plants have been observed by the authors in only a single instance.

Rhizoctonia solani attacks the plants in 2 general areas, at the soil line and at the base of the root system (i.e., root-stem junction). This fungus is extremely aggressive at high soil temperatures (26–30°C), being capable of completely girdling the stem of a rooted plant within 1 week of inoculation. As a root invader, the fungus works from the base of the root system downward; with stem attack, it works from the soil line upward. Therefore a portion of undiscolored stem between the decayed root system and decayed above-ground stem is common with severe *Rhizoctonia* infection, particularly at high soil temperatures. Cases have been observed where the stem has been girdled and all of the upper portions of the root system badly rotted, although the root tips remained white, undecayed, and apparently healthy (Fig. 3-C).

Temperature and root rot development.—The relation of temperature to root rot development is shown graphically in Fig. 4. Within 2 weeks of inoculation with *Thielaviopsis*, most of the root systems were almost completely destroyed at 17°C (Fig. 3-B), destruction being complete in some cases. At 13°, root rot was severe but not as severe as at 17°. At both 21 and 26°, root rot was more severe than at 13°, but very little root rot developed at 30°. In these studies, however, disease was initiated even at 30°, and the fungus was isolated from plants held constantly at this temperature. It appears that *Thielaviopsis* at high temperatures may be somewhat more destructive on poinsettia than was previously believed. Nevertheless, high summer and fall temperatures probably serve to limit development of this disease early in the growing season.

Pythium root rot severity follows the same general pattern as Thielaviopsis root rot in that it is favored by low soil temperature. With *Pythium*, as with *Thielaviopsis*, destruction was more severe at 17°C than at 13°, although the difference was very slight. At 21°, root rot severity dropped considerably but still remained serious. At 26 and 30°, only a trace of Pythium root rot developed, whereas Thielaviopsis root rot was severe at 26°.

The root and stem rots of poinsettia caused by *Rhizoctonia solani* show similar relationships with respect to disease development and temperature. The pathogen did not seem to be aggressive at temperatures below 17°C. At higher temperatures, initiation of infection was very rapid, and disease development increased almost directly with an increase in temperature up to 30°, the highest temperature in the series. At 30°, nearly all of the plants were showing severe root and stem rot symptoms within 2 weeks of inoculation, and the majority were dead. In the 26° series, all plants were severely affected, differing from those in the 30° series only in that the rate of disease development was a little slower. At 21°, destruction was intermediate between that at 26° and 17°; at 13°, only a trace of either root or stem rot developed. The relationships between temperature and fungus growth on PDA, healthy root growth, and disease development are depicted graphically in Fig. 4.

Discussion.—The reported optimum temperature for the growth of *T. basicola* varies from about 29°C (13) down to 21°, as reported here. Undoubtedly some of these differences are due to different techniques used by different workers in growing the organism and determining growth, as well as variations in other environmental factors. Also, the existence of definite physiologic strains has been demonstrated (2, 14, 30), which may explain the differences in part.

According to Lucas (20), the average optimum temperature for 5 isolates of *T. basicola* growing on oatmeal agar was 26°C; growth was measured by colony diameters. When these same isolates grew on potato-dextrose broth and growth was determined by increase in dry weight, the average optimum temperature was 24°C, and more growth occurred at 20° than at 28° or above. When our poinsettia isolate was grown on PDA, the optimum was 24° when growth was determined by measuring colony diameter, and 21°C when determined by dry mycelial weight of the same colonies. The difference was due to the development of denser mycelial mats at the lower temperature.

The optimum temperature for *P. ultimum* was found to be about the same as that reported by Tompkins and Middleton (31), 28°C. All *Pythium* spp. thus far reported from this host have this same optimum. The above authors reported that the optimum temperature for an isolate of *R. solani* from poinsettia was 25°, whereas the isolate used in this study grew best at 30°. This suggests that poinsettia is probably suceptible to both high- and low-temperature "strains" of this fungus.

In a moist soil of neutral reaction, *Thielaviopsis*, *Rhizoctonia*, and *Pythium* are each capable of rendering young healthy plants worthless in a matter of a week if temperature is favorable for the development of the respective diseases. Elucidation of these temperature relationships has been of considerable help in designing a more effective control program in New York.

Keller and Shanks (14) found that, under greenhouse conditions, a 50°F night temperature was more favorable than 60° or 70° for Thielaviopsis root rot development. A night temperature of 70°F delayed infection but did not prevent it. Under the carefully controlled constant-temperature conditions here employed, it was shown that Thielaviopsis root rot developed best at 17°C (63°F) and that root rot was less severe when the plants were grown at 13°C (55°F). It was further shown that constant temperatures as high as 30°C (87°F) did not prevent infection, although root rot was not a serious problem at this temperature. These results are almost identical to those

reported for Thielaviopsis root rot of tobacco (13). The results reported by Keller and Shanks may not be inconsistent with ours, since their average temperatures undoubtedly were higher than their night temperatures.

In general, little attention has been given to the effect of temperature upon the development of Rhizoctonia and Pythium root rots of poinsettia, although these organisms have been known for many years to be of utmost importance (21, 27, 31). Tompkins and Middleton (31) observed that young plants exposed to air temperatures above 80°F or below 60°F become more susceptible to attack. Our results show that Pythium root rot is definitely favored by low soil temperature, 17°C (63°F) being optimum, whereas Rhizoctonia root rot is definitely favored by high soil temperature, 30°C (87°F) being optimum.

With *Pythium*, disease severity is less, whereas with Rhizoctonia, disease severity is greater near the optimum temperature for root growth, even though this also approaches the optimum for the growth of both fungi. With *Thielaviopsis*, disease is increasingly severe as temperature decreases below the optimum for root growth, and the optimum range for disease development includes the lower portion of the optimum range for fungus growth.

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Literature Cited

1. Abdel-Salam, M. M. 1933. Damping-off and other allied diseases of lettuce. J. Pomol. Hort. Sci. **11**: 259-275.
2. Allison, C. C. 1938. Physiologic specialization of Thielaviopsis basicola on tobacco. Phytopathology **28**: 1.
3. Arndt, C. H. 1943. Pythium ultimum and the damping-off of cotton seedlings. Phytopathology **33**: 607-611.
4. Barnett, H. L., V. G. Lilly, and B. M. Waters. 1954. The effects of temperature and method of sugar sterilization on growth of Thielaviopsis basicola. Proc. West Va. Acad. Sci. 1953. **25**: 27-28.
5. Blank, L. M., P. J. Leyendecker, Jr., and R. M. Nakayama. 1953. Observations on black root rot symptoms on cotton seedlings at different soil temperatures. Plant Disease Reptr. **37**: 473-476.
6. Conant, G. H. 1927. Histological studies of resistance in tobacco to Thielavia basicola. Am. J. Botany **14**: 457-480.
7. Dimock, A. W. 1951. Poinsettia trouble a result of root rot. N. Y. State Flower Growers Bull. 69, p. 4-8.
8. Ferris, J. M., B. Lear, A. W. Dimock, and W. F. Mai. 1955. A description of Cornell temperature tanks. Plant Disease Reptr. **39**: 875-878.
9. Flor, H. H. 1930. Relation of environmental factors to growth and pathogenicity of Pythium isolated from roots of sugar cane. Phytopathology **20**: 319-328.
10. Harter, L. L., and W. A. Whitney. 1927. Mottle necrosis of sweet potatoes. J. Agr. Research **34**: 893-914.
11. Houston, B. R. 1945. Culture types and pathogenicity of isolates of Corticium solani. Phytopathology **35**: 371-393.
12. Johann, Helen, J. R. Holbert, and J. G. Dickson. 1928. A Pythium seedling blight and root rot of dent corn. J. Agr. Research **37**: 443-464.
13. Jones, L. R., J. Johnson, and J. G. Dickson. 1926. Wisconsin studies upon the relation of soil temperature to plant disease. Wisconsin Univ. Agr. Expt. Sta. Research Bull. 71, 144 p.
14. Keller, J. R., and J. B. Shanks. 1955. Poinsettia root rot. Phytopathology **45**: 552-558.
15. Khan, A. M. 1950. Temperature and the development of Rhizoctonia solani on legumes. Phytopathology **40**: 14.
16. Lauritzen, J. I. 1929. Rhizoctonia rot of turnips in storage. J. Agr. Research **38**: 93-108.
17. Leach, L. D. 1947. Growth rates of host and pathogen as factors determining the severity of preemergence damping-off. J. Agr. Research **75**: 161-179.
18. LeClerg, E. L. 1941. Comparative studies of sugar-beet and potato isolates of Rhizoctonia solani. Phytopathology **31**: 274-278.
19. Lilly, V. G., and H. L. Barnett. 1951. Ways of measuring growth. *In* Physiology of the fungi. McGraw-Hill Book Co., Inc., New York. p. 27-31.
20. Lucas, G. B. 1955. The cardinal temperatures and pH response of Thielaviopsis basicola. Mycologia **47**: 793-798.
21. Peltier, G. L. 1916. Parasitic Rhizoctonias in America. Illinois Univ. Agr. Expt. Sta. Bull. 189, p. 279-390.
22. Person, L. H. 1944. Parasitism of Rhizoctonia solani on beans. Phytopathology **34**: 1056-1068.
23. Post, K., A. Bing, and F. F. Horton. 1951. Keep poinsettias hot. N. Y. State Flower Growers Bull. 68, p. 5-7.
24. Rawlings, R. E. 1940. Observations on the cultural and pathogenic habits of Thielaviopsis basicola (Berk. and Br.) Ferraris. Ann. Missouri Botan. Garden **27**: 561-598.
25. Richards, B. L. 1923. Soil temperature as a factor affecting the pathogenicity of Corticium vagum on the pea and the bean. J. Agr. Research **25**: 431-449.
26. Samuel, G., and S. D. Garrett. 1932. Rhizoctonia solani on cereals in South Australia. Phytopathology **22**: 827-836.
27. Scheffer, R. P., and W. J. Haney. 1956. Causes and control of root rot in Michigan greenhouses. Plant Disease Reptr. **40**: 570-579.
28. Shanks, J. B., and C. B. Link. 1958. Cultural factors and the incidence of certain soil-borne diseases of poinsettia. Proc. Am. Soc. Hort. Sci. **71**: 522-536.
29. Smith, O. F. 1946. Effect of soil temperature on the development of root canker of alfalfa. Phytopathology **36**: 638-642.
30. Stover, R. H. 1950. The black rootrot disease of tobacco. II. Physiologic specialization of Thielaviopsis basicola on Nicotiana tabacum. Can. J. Research C. **28**: 726-738.
31. Tompkins, C. M., and J. T. Middleton. 1950. Etiology and control of poinsettia root and stem rot caused by Pythium spp. and Rhizoctonia solani. Hilgardia **20**: 171-182.
32. Tompkins, C. M., and J. T. Middleton. 1950. Control of basal rot of cuttings of chrysanthemum and other ornamentals. Bull. Torrey Botan. Club **77**: 287-293.
33. Walker, M. N. 1928. Soil temperature studies with cotton. III. Relation of soil temperature and soil moisture to the soreshin disease of cotton. Florida Univ. Agr. Expt. Sta. S. Gainesville Bull. 197, p. 345-371.
34. Yarwood, C. E. 1946. Isolation of Thielaviopsis basicola from soil by means of carrot disks. Mycologia **38**: 346-348.

OCCURRENCE AND TRANSFER OF A BIOLOGICAL FACTOR IN SOIL THAT SUPPRESSES POTATO SCAB [1]

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SUMMARY

Potato scab is common and often serious in the newly irrigated areas of central Washington, but rare in fields that have been farmed under irrigation for many years. Pot experiments showed that these older farmed soils exert a persistent suppressing effect on scab following inoculation with the pathogen. When heavily infested soil was mixed with equal amounts of suppressing soil, scab was controlled. This effect was lost if the suppressing soil was first steamed. Control of scab in infested soil was good from a combination amendment of 1% suppressing soil and 1% alfalfa meal. Neither constituent was consistently effective alone. A biological factor, possibly microbial, appears to have developed naturally in these older farmed soils, and can be established in other soils by mass soil inoculation fortified with a microbial food source.

Biological suppression of plant-pathogenic organisms in soil through "antagonistic" effects of the associated microflora is believed to be common in cultivated soils (11). Without such a purifying action by beneficial microorganisms, the soils of the world would quickly become so infected with root-disease organisms that plant growth would be seriously threatened. These biological mechanisms offer intriguing possibilities for improved crop disease control, but not enough is yet known about the organisms involved or their mode of action to use them specifically on a field scale for this purpose.

It has long been known that, under certain conditions, green manure treatments or particular crop sequences sometimes suppress potato scab, caused by *Streptomyces scabies* (Thaxt.) Waksman & Henrici. In 1926 Sanford (9) suggested that this type of control might be due to stimulation, by organic residues, of organisms antagonistic to the pathogen. A specific example was soon demonstrated by Millard and Taylor (5), who found that a saprophytic actinomycete inhibited scab when added to organic residues incorporated in sterilized inoculated soil. Since then there have been numerous reports of scab suppression by organic soil treatments (6, 8, 10, 11), or, in sterile-soil experiments, by the use of specific antagonistic organisms (1, 2).

Such experimental control of scab has resulted from incorporating large amounts of organic matter in the soil, or inoculating it heavily with antagonists. A temporary surge in microbial activity following such treatment creates a microflora greatly different in kind and population density from the previous equilibrium. If, however, scab suppression is naturally established at the lower microbial levels present in unamended soils, it should be more lasting than one induced by the sudden addition of energy-rich substrates.

The work reported here concerns a study of such a situation in soils of central Washington, leading to the conclusion that some scab-free soils have an apparent biological suppressing effect on the disease, and that this suppression can be transferred to infested soil, thereby reducing scab incidence.

NATURAL OCCURRENCE OF SCAB SUPPRESSION.—An erratic distribution of potato scab in soils of similar type in central Washington has become obvious in recent years following the expansion of irrigated acreage. Older irrigated potato-producing areas are almost scab-free, whereas adjacent lands irrigated and cropped only in the last 15 years or so have become generally infested with the pathogen. The difference in scab incidence has been striking where new land has been farmed adjacent to old irrigated fields. In several such cases where continuous potato production has been attempted, the new-land crop has become uniformly scabby, although scab failed to appear in the old land.

No records have been found that show whether scab was a problem in these older areas when they were first settled, over 50 years ago, but some older farmers have recalled that this was the case. There is no consistent cropping history in these instances. Alfalfa has been a frequent crop, along with sugar beets, corn, cereals, and potatoes.

It is safe to assume that these fields that have produced many crops of potatoes have been contaminated, at least occasionally, with the scab organism. There is evidence that the pathogen exists in the virgin soil (3, 7), and since seed treatment has seldom been used, the seed must have carried the organism quite frequently. Also, soil and associated organisms often move from farm to farm by means of drainage water, wind storms, and farm equipment. Absence of scab in these cases must therefore be due to conditions unfavorable for the organism or its parasitism, not to mere escape.

COMPARISON OF SCABBING TENDENCY OF OLD AND NEW SOILS.—When these studies were begun it seemed possible that soil type might have a bearing on the occurrence of scab, especially since there is a wide

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TABLE 1.—*Effect of cropping history on survival of potato scab for a 5-year period following inoculation in 12 soils from the Columbia Basin*

	Scab index					Average
	1953	1954	1955	1956	1957	1954–57
Virgin soils						
Ritzville	2.2	5.5	2.0	7.8	4.5	5.0
Timmerman	3.8	12.4	5.4	23.1	18.7	14.9
Ephrata	2.6	15.2	7.4	25.7	12.6	15.2
Sagemoor #1	2.9	9.9	9.0	14.7	19.0	13.2
Sagemoor #2	2.5	8.9	12.2	19.2	10.3	12.7
Esquatzel	2.3	12.0	7.1	16.9	17.3	13.3
Wheeler	4.1	17.7	12.2	19.7	10.5	15.0
Warden	3.1	4.5	1.8	27.8	2.7	9.2
Burke	2.1	14.6	8.8	32.3	25.5	20.3
Cultivated soils						
Sagemoor #3	1.0	0.4	0.2	0.6	1.1	0.6
Naches	2.1	0.5	0.5	0.5	0.5	0.5
Sagemoor #4	2.9	0.2	0.2	0.7	0.3	0.4

range of soil pH and calcium carbonate in the soils of central Washington. Previous work had shown that a high soil calcium content at a soil pH above 8.0 resulted in a reduction in scab incidence (4). Accordingly, large samples of surface soil were obtained from virgin areas of nine different soil types in central Washington. Similar samples were selected from farm fields with a record of scab-free potato production in recent years, in 3 old-farmed areas of the Yakima Valley.

The experiment was set up out-of-doors in bottomless 9×9-in. metal containers placed 11 in. deep in soil with a 2-in. rim remaining above the soil line. Each soil sample was screened, mixed, and distributed into 4 containers in a randomized block design. For the first potato crop, scab-infested seed pieces of the White Rose variety were planted. In succeeding years, scab-free seed treated with acidified mercuric chloride was used for each crop. Each year the tubers were harvested at normal maturity and graded into scab classes by the following scheme: 0, no scab; I, < 1% of surface scabbed; II, 1–5%; III, 6–10%; IV, 11–25%; V, 26–50%; VI, 51–75%; VII, 76–100%.

Grading was simplified and standardized with prepared charts that showed ovals with various percentages of their area blackened out to simulate scab lesions. By using the arithmetical mean for each class, a Scab Index was calculated for each replicate of each soil.

During the 5 years of this experiment, no precautions were taken to prevent accidental contamination of the soils from wind-blown soil or through handling, except that tools were wiped free of loose soil before use. Irrigation water from the valley canal system was used when needed. Low rates of N fertilizer were added annually.

Table 1 summarizes the results for 5 successive crops of scab-susceptible White Rose potatoes. The first-year crop, produced from scab-infected seed pieces, was slightly scabby in all soils, indicating that inoculum was successfully introduced, even into the old-farmed soils. In succeeding years, scab damage increased to a very high level in all the nine virgin soils samples, but dropped down to little more than a trace in the three old-farmed soils, though there was enough to demonstrate that the pathogen was still present at the end of five crops. Its parasitism, or population level, however, was consistently suppressed.

COMPARISON OF CHEMICAL PROPERTIES OF SOILS.—During the last season of the above experiment, after the soils had been cropped uniformly to potatoes for five years and otherwise treated the same, the soils were sampled for chemical analysis. Since differences

TABLE 2.—*Chemical characteristics of Columbia Basin soils listed in Table 1*

Soil	Cation exchange capacity[a] (me/100 g)	Exchangeable K[a] (me/100 g)	Exchangeable Na[a] (me/100 g)	$CaCO_3$ Equivalent[b] (%)	ECx 10^3[a] (mmhos/cm)	pH[c]	O.M.[d] (%)
Virgin soils							
Ritzville	12.6	2.0	0.23	1.1	0.73	7.4	0.52
Timmerman	12.2	2.1	0.19	0.1	0.57	6.6	0.58
Ephrata	12.4	2.1	0.23	0.1	0.58	6.8	0.69
Sagemoor #1	14.0	1.9	0.27	1.6	0.80	7.2	0.62
Sagemoor #2	13.4	2.3	0.24	1.3	0.70	7.5	0.23
Esquatzel	12.4	2.0	0.22	0.3	0.70	7.5	0.67
Wheeler	12.0	2.1	0.21	0.8	0.69	7.5	0.43
Warden	15.3	2.3	0.27	0.1	0.52	6.4	0.36
Burke	11.6	1.7	0.19	0.3	0.63	7.3	0.70
Average	12.9	2.1	0.23	0.6	0.66		0.53
Cultivated soils							
Sagemoor #3	12.2	1.9	0.22	0.4	0.52	7.3	0.80
Naches	11.1	1.6	0.19	0.2	0.72	7.0	0.56
Sagemoor #4	18.2	2.7	0.30	0.2	0.80	6.2	1.08
Average	13.8	2.1	0.24	0.3	0.68		0.81

[a] Exchange capacity, exchangeable cations, and conductivity of saturation paste extract (ECx 10^3) were determined according to methods in USDA Handbook No. 60.

[b] $CaCO_3$ equivalent, by measuring the CO_2 evolution from addition of 6*N* HCl to the soil in a Scheibler lime apparatus.

[c] Soil pH, determined on saturated paste with a Beckman glass-electrode meter.

[d] Organic matter, by Melsted's modification of the Allison-De Turk dichromate method. These analyses were performed by Carl L. Crawford, physical science aid.

TABLE 3.—*Effect of mixtures of infested and scab-suppressing soil, with and without steaming, on incidence of scab on potatoes (each figure an average of 4 replications)*

Treatment	Scab index	Scabby tubers[a] (%)
Naturally infested soil (NI)	1.4	37.1
Scab-suppressing soil (SS)	0.1	0.0
NI + 10% SS	1.5	43.9
NI + 50% SS	0.3	0.0
NI + 90% SS	0.2	0.0
NI + 10% steamed SS[b]	1.2	22.7
NI + 50% steamed SS	1.5	36.0
NI + 90% steamed SS	0.7	11.8

[a] Tubers with only a few inconspicuous lesions were considered non-scabby.
[b] Suppressing soil autoclaved 2 hours at 15 lb. pressure.

in scabbing were still strongly expressed, any chemical differences specifically influencing the disease should be more clearly revealed than they would have been in samples taken at the start of the experiment. Samples were composited from the 4 replicates of each soil, and dried and screened before analysis.

The analyses (Table 2) reveal no differences clearly associated with occurrence of scab. Most of these soils are classed as fine sandy loams, low in organic matter, and neutral or slightly alkaline in reaction, conditions usually conducive to potato scab.

TRANSFER OF SUPPRESSING EFFECT TO INFESTED SOIL.—The first experiment showed that the scab suppression suspected in the old-farmed areas was real, and that it was not upset by the somewhat unnatural environment and handling in the small containers. The next step was to find out if the suppressing factor could be destroyed by steaming, and whether it could be transferred to a naturally infested soil.

A sample of soil from the scab-suppressing field Sagemoor #1 (Table 1) was passed through a ⅛-in.-mesh screen to break up lumps. Another sample, from a nearby newly irrigated field heavily infested with scab, was prepared similarly. Part of the suppressing soil was then steamed 2 hours at 15 lb. pressure. These soils were then mixed in various proportions, divided into four 5-gal. containers per treatment, and planted with scab-free White Rose potatoes. The results (Table 3) show that unsteamed suppressing soil almost completely prevented scab when added at rates of 50% and 90%, but not at 10%. When suppressing soil was steamed, this effect was lost. The reduced scab at the 90% level of steamed soil may be a dilution effect, or it may indicate a different and temporary type of scab suppression following recolonization of the steamed soil by an antagonistic microflora.

USE OF AN ALFALFA FOOD SOURCE IN TRANSFER OF SUPPRESSION.—General experience in attempting to introduce and establish new microorganisms in unsterile soil has been unsatisfactory unless an extra microbial food source is added with the inoculum. Experiments were therefore made to test the value of alfalfa meal in reducing the amount of soil needed. Alfalfa was chosen because it is the most common green manure crop used in this area. Virgin Ritzville fine sandy loam was used, treated with various rates of suppressing soil and alfalfa meal, and then uniformly inoculated with *S. scabies*. Inoculum was prepared from scabby potato peelings passed through a meat grinder and allowed to dry slightly before being mixed into the treated soils. This experiment was arranged out-of-doors in 18-in.-diameter concrete tile sections set in the ground. Four tiles were used for each treatment. The bottom portions of these tiles were filled with field soil. The treated soils were placed in a 10-in. layer on top of this soil. This method was used because of the large volume of treated soil that would be required to fill the whole tile. From previous experiments it was assumed that the 10-in. layer of soil would surround the tubers and that the growth of roots into the untreated soil would not affect the scab reaction. This assumption was apparently sound.

Three consecutive crops of White Rose potatoes were grown in these soils. The second crop was produced in the greenhouse by transferring the top 4 in. of soil from the tiles to 5-gal. containers. After the second crop, the soil was returned to the original tile and a third crop planted. In this way, three crops were obtained in two growing seasons.

The scab incidence is reported in Table 4, both as scab indices and as percentage of tubers scabby. In the latter case, minute and rare lesions were disregarded, and tubers falling in Class O and I were considered non-scabby. The combination treatment of 10% suppressing soil with 1% alfalfa meal gave a scab index of 0.7 over a three-crop average, compared with 0.3 for 100% suppressing soil, and 9.0 for non-suppressing soil. Alfalfa meal alone permitted as much scab as occurred in the non-suppressing soil, whereas the 10% suppressing soil treatment was intermediate in reaction. This treatment had been completely ineffective in the previous experiment (Table 3). Again, steaming destroyed any scab-suppressing effect. Although an average of 14.3% of the tubers

TABLE 4.—*Transfer of scab-suppressing effect from a scab-free soil to virgin inoculated soil*

Treatment	1st crop	2nd crop	3rd crop	Av.
	Scab index			
Suppressing soil (SS)	0.4	0.4	0.3	0.3
Suppressing soil steamed	2.8	13.3	8.9	8.3
Non-suppressing soil (NSS)	0.6	19.4	6.9	9.0
NSS + 10% SS	0.5	2.9	3.3	2.2
NSS + (10% SS steamed)	1.8	12.6	13.2	9.2
NSS + 10% SS + 1% alfalfa meal	0.3	1.0	0.9	0.7
NSS + 1% alfalfa meal	1.8	13.3	12.1	9.1
NSS (steamed)	3.0	31.2	12.5	15.6
	% scabby tubers			
Suppressing soil (SS)	3.9	1.0	2.9	2.6
Suppressing soil steamed	43.6	84.2	73.3	67.0
Non-suppressing soil (NSS)	9.0	100.0	78.1	62.4
NSS + 10% SS	6.3	55.0	49.0	36.8
NSS + (10% SS steamed)	23.0	96.0	94.1	71.0
NSS + 10% SS + 1% alfalfa meal	0.3	21.7	21.0	14.3
NSS + 1% alfalfa meal	32.4	84.6	83.8	66.9
NSS steamed	52.7	100.0	94.9	82.5

graded scabby in the combination treatment, most of them had such inconspicuous lesions that they would have been disregarded in commercial grading. Representative tubers from this treatment are shown in Fig. 1–8.

CONTROL ATTEMPTS WITH INOCULATED ALFALFA.—The above experiments supported the hypothesis that a biological factor was involved and that its introduction into infested soil was possible when accompanied by an added food source. However, if this transfer of suppression has any possibility for practical field use, lower rates of inoculum and food source must be effective. Attempts were therefore made to obtain the same effect by inoculating the alfalfa meal with a slurry of the scab-suppressing soil. The slurry was made from 8 lb. of soil in 3 gal. water. The alfalfa meal was weighed out, and then moistened with the slurry to a damp consistency. It was then thoroughly mixed into naturally scab-infested soil. The pots were planted with White Rose potatoes 1 week after the treatments were made.

Two crops were grown in this experiment, one in the greenhouse and one outdoors. The results (Table 5) again demonstrate a reduction in scab by suppressing soil and alfalfa meal. The pronounced scab control resulting from the 1% alfalfa treatment is contrary to previous results. Since this treatment resulted in less scab than the same amount of alfalfa slurry-treated or used with 1% suppressing soil, an experimental error is strongly suspected. Assuming this error, one can conclude that using 1% suppressing soil in conjunction with alfalfa is almost as effective as using 10%, and that even slurry-inoculated alfalfa is effective at the 1% rate but not at 0.1 or 0.01%. In view of the uncertainty raised by the performance of alfalfa alone, however, this conclusion must be tentative.

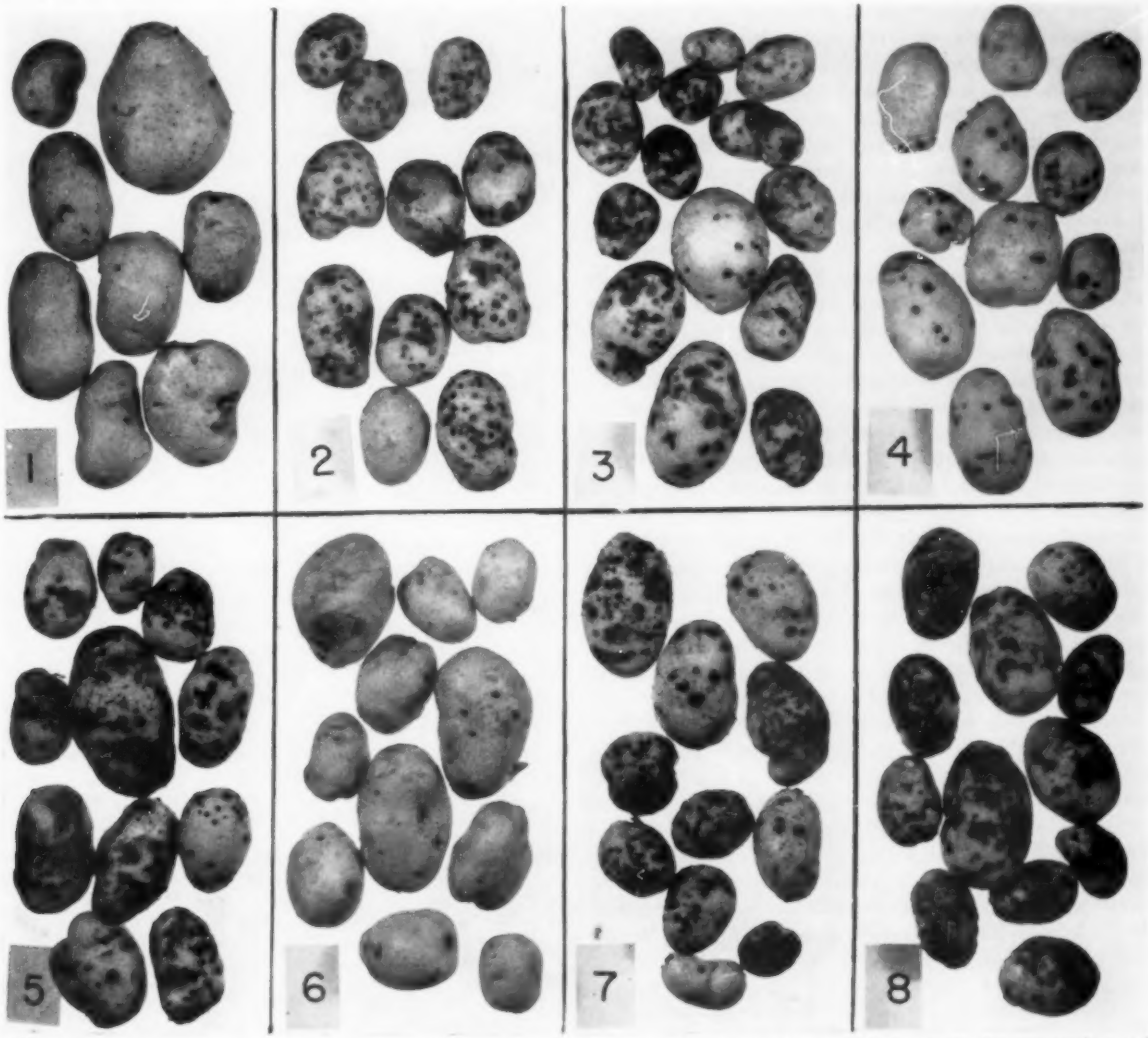

Fig. 1–8. Representative tubers from treatments of scab-infested soil with various combinations of suppressing soil and alfalfa meal (Table 4, second crop). **1)** Suppressing soil (SS); **2)** suppressing soil steamed; **3)** non-suppressing soil (NSS); **4)** NSS + 10% SS; **5)** NSS + 10% SS steamed; **6)** NSS + 10% SS + 1% alfalfa meal; **7)** NSS + 1% alfalfa meal; **8)** NSS steamed.

TABLE 5.—*Scab suppression in naturally infested soil by alfalfa meal inoculated with suppressing soil (each figure an average of 4 replications)*

Treatment	Scab index		% scabby tubers		
	1st crop	2nd crop	1st crop	2nd crop	Soil pH*
Untreated infested soil (NI)	4.9	2.6	83.3	40.4	8.0
Suppressing soil (SS)	0.0	0.5	0.0	0.0	8.1
NI + 1% alfalfa + 10% SS	1.2	0.4	17.8	4.7	7.5
NI + 1% alfalfa + 1% SS	0.7	1.2	13.0	18.2	8.1
NI + 1% slurry treated alfalfa	2.1	0.6	19.0	14.3	8.1
NI + 0.1% slurry treated alfalfa	6.6	1.9	72.7	35.8	8.1
NI + 0.01% slurry treated alfalfa	2.5	2.2	40.0	33.3	8.0
NI + 1% untreated alfalfa	0.4	0.4	4.2	8.3	8.0

* Taken after harvest of second crop.

DISCUSSION.—These experiments demonstrate a scab-suppressing factor in several soils that could not be correlated with any obvious chemical or physical soil properties. The suppression is dominant over inoculum when infested and suppressing soils are mixed in equal amounts, but is destroyed by steaming. This effect does not seem to be a temporary effect from organic residues, because it persisted over a 5-year period of continuous potato cropping. During this time, a low level of scab occurred each year, showing that the pathogen was present and that freedom from scab was not due to a failure of inoculation the first year.

Some success was obtained in controlling scab in naturally infested soil by amendments of suppressing soil and alfalfa meal. A clear-cut scab reduction that persisted through 3 consecutive crops was obtained from amendments of 10% suppressing soil and 1% alfalfa meal in 1 experiment. Results were similar for 2 successive crops in a second experiment. Inconsistencies and variability in the data make it difficult to evaluate the effect of alfalfa meal used as an amendment without suppressing soil. In one experiment this treatment increased scab, but in the second it resulted in less scab than the combination treatment.

Suppressing soil used at the rate of 10% without alfalfa was generally without sufficient effect to give any practical control of scabbing. Nevertheless, it was not entirely ineffective, and such differences as can be seen between Fig. 3 and Fig. 4 emphasize the scab suppression that occurs naturally in some soils. Furthermore, the unsteamed suppressing soil did not produce scab even when mixed with as much as 50% infested soil. The significance of this is apparent when one considers the standard assumption that field soil can be easily infested with *S. scabies* by dust, irrigation water, seed, or contaminated implements. In other experiments (unpublished) we have established the scab organism in virgin ground by dusting potato seed pieces with infested soil at a rate of 20 lb./A. The dominance of a known suppressing soil over the infested soil, and the susceptibility of the suppressing factor to steaming, point more strongly to a biological factor than to any chemical or physical one. Although the data do suggest that the suppression factor is biological, they do not indicate whether its effect is directly upon the pathogen in the soil, upon its pathogenicity, or upon the susceptibility of the host tubers.

Much attention has been given to studying the factors responsible for destructive outbreaks of root disease in crop plants. In the case of organisms that, like *S. scabies*, are capable of saprophytic survival in a wide variety of soils, it may be equally important to investigate in detail the natural factors that prevent these diseases from being even more widespread and damaging. It is generally assumed that cultivated soils sometimes develop microbial equilibria that have a controlling effect on soil-borne pathogens, and that soil management practices such as green manure treatments or rotations are important because of their influence on these equilibria. The experiments reported here suggest that a disease-controlling equilibrium, which may take many years to appear naturally, may be induced in one season by appropriate treatments. A thorough understanding of the processes and organisms involved may eventually lead to their direct utilization for disease control.

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LITERATURE CITED

1. DAINES, R. H. 1937. Antagonistic action of Trichoderma on Actinomyces scabies and Rhizoctonia solani. Am. Potato J. **14**: 85-93.
2. GOSS, R. W. 1937. The influence of various soil factors upon potato scab caused by Actinomyces scabies. Nebraska Agr. Expt. Sta. Research Bull. **93.**
3. LUTMAN, B. F. 1923. Potato scab in new land. Phytopathology **13**: 241-244.
4. MENZIES, J. D. 1950. Potato scab control with calcium compounds. (Abstr.) Phytopathology **40**: 968.
5. MILLARD, W. A., AND C. B. TAYLOR. 1927. Antagonism of micro-organisms as the controlling factor in the inhibition of scab by green manuring. Ann. Appl. Biol. **14**: 202-215.
6. OSWALD, J. W., AND O. A. LORENZ. 1956. Soybeans as a green manure crop for the prevention of potato scab. (Abstr.) Phytopathology **46**: 22.
7. PRATT, O. A. 1918. Soil fungi in relation to diseases of the Irish potato. J. Agr. Research **13**: 73-100.
8. ROUATT, J. W., AND R. G. ATKINSON. 1950. The effect of the incorporation of certain cover crops on the microbial balance of potato scab infested soil. Can. J. Research C. **28**: 140-152.
9. SANFORD, G. B. 1926. Some factors affecting the pathogenicity of Actinomyces scabies. Phytopathology **16**: 525-547.
10. SANFORD, G. B. 1946. Soil-borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Sci. **61**: 9-21.
11. WOOD, R. K. S., AND M. TVEIT. 1955. Control of plant diseases by use of antagonistic organisms. Botan. Rev. **21**: 441-491.

DEMONSTRATION OF PHYSIOLOGICAL RACES WITHIN THREE ROOT-KNOT NEMATODE SPECIES ATTACKING ALFALFA[1]

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SUMMARY

A test of the virulence of 20 collections of root-knot nematodes (*Meloidogyne* Goeldi, 1887) was made on five alfalfa host differentials. Three biotypes were found in *M. incognita acrita*, 2 in *M. javanica javanica*, and 2 in *M. hapla*. A single alfalfa clone selected from the variety Vernal gave resistance to all collections.

Behind a facade of phenotypic uniformity, the microscopic obligate parasites known as root-knot nematodes successfully evaded taxonomic subdivision until only recently. However, Christie and Albin (3), in 1944, and Christie and Havis (4), in 1948, experimentally demonstrated the existence of races within the root-knot nematodes, then regarded as one species, *Heterodera marioni*. In 1949, Chitwood (2) placed the root-knot nematodes in the genus Meloidogyne, and described several species within this genus. The above work was very useful in explaining many previous inconsistencies of field and laboratory results.

Subsequent work by Allen (1) indicated that variation within a species of root-knot nematode (*Meloidogyne incognita acrita* Chitwood, 1949) was even greater than previously believed. He found that several populations of this species of root-knot nematode collected from cotton in California displayed a wide diversity in host-plant specificity. Martin (9) found physiologic races of both *M. incognita* and *M. incognita acrita* on cotton, while Sasser and Nusbaum (10) found races of *M. incognita acrita* differing in pathogenicity on tobacco.

Lider (8) found considerable variability in the reaction of two species of *Vitis* to diverse collections of *M. incognita acrita*. This indicated that, within this single species of root-knot nematode, there existed racial differences in ability to attack *Vitis* spp.

The existence of races has also been reported for nematodes other than root-knot nematodes. Smith (11), in 1951, reported that at least 2 distinct biologic races of the stem nematode, *Ditylenchus dipsaci*, may attack alfalfa. Similarly, Jones (7) clearly demonstrated the existence of races within the potato root eelworm, *Heterodera rostochiensis* Woll.

Three species of root-knot nematodes are known to attack alfalfa in California. Their prevalence and damage in alfalfa fields is becoming of economic concern. Preliminary to any breeding program, it would be invaluable to determine if races of the parasite existed within the three species of root-knot nematodes that attack alfalfa. This report summarizes the results of such an investigation.

MATERIALS AND METHODS.—Twenty-four different collections of root-knot-infected alfalfa plants were obtained in the summer of 1956, representing a diverse sampling of the alfalfa-growing areas of California.

For each collection, the infected alfalfa roots were finely chopped, mixed thoroughly into steam-sterilized soil, and replicated by placing equal portions in three 7-in. pots. The pots were then partly submerged in a bench filled with soil containing a soil-heating cable with a thermostatic control. The soil temperature was maintained at 28°C for optimum nematode activity and reproduction. Seed of a susceptible tomato variety (Pearson's Improved) was then sown in each pot to increase the nematode populations. Each population was allowed to reproduce for 7 months, after which the plant tops were removed, leaving the heavily infected roots intact in the soil. This series of infested soils (three 7-in. pots of each) was then planted with the 5 alfalfa host differentials to determine if physiological strains of the nematode existed.

The 5 plant types used as host differentials in this investigation are listed in Table 1, with relevant information for each. The host differentials were plants of 5 alfalfa varieties, chosen for varied genetic backgrounds and expected root-knot reactions (Table 1).

The first 3 host differentials were cloned by placing vegetative stem cuttings from these plants in steam-

TABLE 1.—*Alfalfa host differentials used in tests against 20 root-knot nematode collections*

Host differential	Variety source	Method of propagation	Expected root-knot reaction
Africa	Africa	clone of one plant	*M. javanica javanica* resistant *M. hapla* susceptible
M-8	Hilmar	clone of one plant	*M. hapla* resistant
M-9	Vernal	clone of one plant	*M. hapla* resistant
Caliverde	Caliverde	open-pollinated seedlings	*M. hapla* susceptible
JR-7	California Common	self-pollinated seedlings	Segregating for resistance to *M. javanica javanica*

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sterilized sand and leaving them in this medium until properly rooted (3–4 weeks). Seed from host differentials 4 and 5 was planted into flats with steam-sterilized soil and allowed to grow until the seedlings were well established (4–6 weeks). A cutting or seedling from each of these selected alfalfa sources was then transplanted to each of the three 7-in. pots representing each collection. Seed of a susceptible variety of tomato (Pearson's Improved) was also planted in each pot to serve as an index of nematode concentration and activity. Precautions were taken against contamination from soil of adjacent collections by spacing each soil series (3 pots) 6 in. from one another and by careful watering.

The plants were grown for 3½ months, then carefully removed from the pots and examined for degree of infection. All roots were examined with a stereoscopic microscope and classified as resistant or susceptible, based on the criterion of reproduction of nematodes (mature females with egg masses).

The whole root of a well-infected alfalfa plant (or tomato root, if no alfalfa plants were infected) was taken from each collection at the time of classification, and the nematode species determined.

EXPERIMENTAL RESULTS.—Four of the 24 original collections failed to show adequate infection on the susceptible tomato check plants, and hence were discarded. All of the remaining 20 collections showed heavy infection on tomato. The reaction of some of these collections to alfalfa is recorded in Table 2.

The genetically uniform clones of Africa, M-8, and M-9 are critical for distinguishing differential reactions among the various nematode collections. Each of these clones is uniform, whereas Caliverde was from heterogeneous open-pollinated seed, and JR-7 was from segregating self-pollinated seed.

One of the most significant results in Table 2 is the differential reactions exhibited by collections Caruthers-4 and Arvin-6. The Caruthers-4 collection reproduced freely on Africa, whereas all other collections failed to reproduce on this clone. Similarly, the Arvin-6 collection was unique in failing to reproduce on clone M-8. This variability of host-pathogen relationship clearly indicates the occurrence of biotypes of races within *M. incognita acrita*.

TABLE 2.—*Reaction of 8 collections of Meloidogyne incognita acrita on differential hosts of alfalfa*

Locality of collection	Alfalfa differential hosts				
	Africa	M-8	M-9	Caliverde	JR-7
Atwater	–[a]	+[b]	–	+, –[c]	+, –[d]
Caruthers-3	–	+	–	+	+, –
Caruthers-4	+	+	–	+	+, –
Caruthers-5	–	+	–	+	+, –
Fowler-1	–	+	–	+	+, –
Fowler-2	–	+	–	+	+, –
Fresno	–	+	–	+, –	+, –
Arvin-6	–	–	–	+, –	–

[a] + indicates egg masses present.
[b] – indicates no egg masses or apparent infection.
[c] Open-pollinated seed, hence plants heterogeneous for root-knot reaction.
[d] Selfed progeny segregating resistant and susceptible.

The results of the tests involving the 20 collections did not indicate any variation among the 9 collections of *Meloidogyne javanica javanica* Treub, 1885. Information obtained from additional studies, however, strongly suggests that physiological races exist within *M. javanica javanica* also. For example, clone M-8 was susceptible to all the collections of *M. javanica javanica* included in this study. However, S_2 (second-generation selfed progeny) seed from a selected S_1 plant of M-8 was tested against an *M. javanica javanica* population at Riverside, California,[5] and of 17 plants tested against this inoculum, 10 were resistant and 7 were susceptible. The selfed progeny of 2 other selections, M-4 and M-9, were tested against the Riverside population of *M. javanica javanica*, and they also gave a majority of resistant plants (7 resistant to 4 susceptible, and 7 resistant to 2 susceptible, respectively). The predominance of resistant plants in the selfed progenies of these 3 selections indicates that resistance is dominant. Therefore, the resistance appearing in the S_2 family of M-8 must have been derived from a dominant gene present in the original M-8 selection. Thus it can be logically inferred that the Riverside collection of *M. javanica javanica* would not have been able to reproduce on the original clone of M-8. This is in striking contrast to all the *M. javanica javanica* collections tested at Davis, which reproduced freely on this clone. This variability in reaction of M-8 to different collections of *M. javanica javanica* strongly indicates the occurrence of races within this species.

Only 2 collections of *M. hapla* Chitwood, 1949, were tested, and both reacted similarly on the alfalfa host differentials. Additional genetic data, however, clearly revealed that these 2 collections are different biological races of one species of root-knot nematode. In tests of the selfed progeny of a selection of Vernal (M-4), chosen because of its demonstrated resistance to Caruthers-1 collection of *M. hapla*, 273 resistant and 4 susceptible plants were observed. In contrast, when selfed progeny of M-4 were tested against the Arvin-1 collection of *M. hapla*, all of the plants observed were completely susceptible to this collection of nematode. Thus races also exist within the *M. hapla* species of root-knot nematode.

The only information obtained from the Caliverde host differential is that some plants within this alfalfa variety are resistant to some of the collections of *M. javanica javanica* and *M. incognita acrita*. Similarly, selection JR-7 contains an inherent resistance against all of the collections of these 2 species of root-knot nematodes. Both Caliverde and JR-7 are, however, completely susceptible to the 2 collections of *M. hapla* included in this test.

From a practical plant-breeding standpoint, the most important result from this study was the observation that clone M-9 was resistant to all of the 20 col-

[5] This test was conducted by Dr. I. J. Thomason, Department of Plant Nematology, University of California, Riverside.

lections of root-knot nematodes (Table 2). Resistant plants also appeared when S_1 seed was tested against the Riverside population of *M. javanica javanica*. Hence the resistance of M-9 held up against all root-knot nematode collections in California tested in this study. This is particularly noteworthy since these collections included 3 species of *Meloidogyne* spp. as well as recognized races within each of these species.

DISCUSSION AND CONCLUSIONS.—The occurrence of different physiological subunits within *Meloidogyne* spp. was unrecognized until relatively recently (1, 2, 3, 4, 8, 11). A classical example may be cited from work with peaches. In 1943, Weinberger, Marth, and Scott (12) tested several introductions of peach seedlings for root-knot resistance and found 2 selections, Yunnan and Shalil, resistant to the nematodes used in their test. In 1950, however, Havis et al (5) found that, although *M. incognita acrita* was ineffective in attacking these 2 peach rootstock selections, *M. javanica javanica* was virulent. They later found a selection that displayed a high degree of resistance to both of these nematodes. Similar findings, at the subspecies or race level, have been recorded by Jones (7). He reported numerous biotypes within the potato root eelworm, *Heterodera rostochiensis*, that were capable of infecting resistant clones of *Solanum* spp. This work clearly demonstrated the futility of using a very limited collection of nematodes in breeding for resistance. Both examples illustrate the importance of developing varieties of crops with resistance to a wide collection of species and races of the parasite involved.

From these investigations it was conclusively demonstrated that different biotypes or physiological races existed within *M. incognita acrita* and *M. hapla*. Through less direct means, it was also concluded that *M. javanica javanica* also contained different physiological races with distinct host preferences. The detection of races within each of these species of nematodes attacking alfalfa should caution the breeder to use extensive and diverse sources of inoculum in developing a resistant variety.

In breeding for resistance to any pathogen it is important for the plant breeder to have information on the physiologic races or biotypes to which his resistant varieties may be subjected in the field. Identification of races is dependent on establishing an appropriate series of differential hosts. In the case of self-pollinated crops such as wheat, in which varieties may be maintained as essentially pure lines, the seed progeny of a variety may be quite satisfactory as differentials if precautions are taken to maintain the purity of the variety. With a cross-pollinated crop such as alfalfa, however, the "purity concept" is untenable, for varieties are composed of individuals with considerable heterogeneity for many characters; due to its breeding behavior, the genetic base of a cross-pollinated variety is necessarily much broader than the genetic base of a self-pollinator. In the present study, the variety Vernal has yielded individuals resistant to two distinct races of *M. hapla*, individuals resistant to one race but not the other, and individuals susceptible to both races. In a small sample of individuals, as is frequently used for racial testing, this variety then might give a resistant, susceptible, or mixed reaction to the same race. In the present experiment the variety Caliverde was not useful in differentiating races, because of the mixture of genotypes.

Alfalfa can be cloned and propagated indefinitely by vegetative means. The clones are of constant genetic composition. Clones M-9, M-8, and M-4 and the clone of Africa proved to be satisfactory differentials in the present study. It is therefore proposed that these clones be maintained to serve as a basis in future studies of physiologic races. As additional studies are made, additional differentials may be added. This same principle of standardizing host differentials could be extended to other crops to facilitate positive verification of species and races of nematodes by the use of similar host differentials.

The problem of races among different nematode collections is quite different from the race problem in certain diseases. The spread of a particularly virulent race of air-borne fungal disease is phenomenal. Many such examples have been recorded from stem rust of wheat (6). The rate of spread of virulent biotypes of nematodes, however, is very slow in comparison with stem rust, because of the lack of mobility of the parasite. Thus, if a crop variety has been developed resistant to a large number of nematode collections taken from the main areas of proposed use, the variety should "stand up" for a considerable period.

The genetic behavior of resistance in alfalfa against *M. hapla* and *M. javanica javanica* has been determined, and will be reported in a later paper.[6]

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LITERATURE CITED

1. ALLEN, M. W. 1952. Observations on the genus Meloidogyne Goeldi 1887. Proc. Helminthol. Soc. Wash. D. C. **19**: 44-51.
2. CHITWOOD, B. G. 1949. Root-knot nematodes. I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminthol. Soc. Wash. D. C. **16**: 90-104.
3. CHRISTIE, J. R., AND F. E. ALBIN. 1944. Host-parasite relationships of the root-knot nematode, Heterodera marioni. I. The question of races. Proc. Helminthol. Soc. Wash. D. C. **11**: 31-37.
4. CHRISTIE, J. R., AND L. HAVIS. 1948. Relative susceptibility of certain peach stocks to races of the root-knot nematode. U. S. Dept. Agr. Plant Disease Reptr. **32**: 510-514.
5. HAVIS, L., B. G. CHITWOOD, V. E. PRINCE, G. S. COBB, AND A. L. TAYLOR. 1950. Susceptibility of some peach rootstocks to root-knot nematodes. U. S. Dept. Agr. Plant Disease Reptr. **34**: 74-77.
6. JOHNSON, T., AND G. J. GREEN. 1957. Physiologic specialization of wheat stem rust in Canada, 1919 to 1955. Can. J. Plant Sci. **37**: 275-287.
7. JONES, F. W. G. 1957. Resistance-breaking biotypes of the potato root eelworm (Heterodera rostochiensis Woll.). Nematologica **2**: 185-192.
8. LIDER, L. A. 1954. Inheritance of resistance to a root-knot nematode (Meloidogyne incognita var. acrita Chitwood) in Vitis spp. Proc. Helminthol. Soc. Wash. D. C. **21**: 53-60.
9. MARTIN, W. J. 1954. Parasitic races of Meloidogyne

[6] Coplen, B. P., and E. H. Stanford. Unpublished data.

incognita and M. incognita acrita. Plant Disease Reptr. Suppl. **227**: 86-88.
10. SASSER, J. N., AND C. J. NUSBAUM. 1955. Seasonal fluctuations of host specificity of root-knot nematode populations in two-year tobacco rotation plots. Phytopathology **45**: 540-545.
11. SMITH, O. F. 1951. Biologic races of Ditylenchus dipsaci on alfalfa. Phytopathology **41**: 189-190.
12. WEINBERGER, J. H., P. C. MARTH, AND D. H. SCOTT. 1943. Inheritance study of root-knot nematode resistance in certain peach varieties. Proc. Am. Soc. Hort. Sci. **42**: 321-325.

STREAK-INCITING VIRUSES OF CANNING PEA[1]

W. S. Kim and D. J. Hagedorn

SUMMARY

A collection of streak-diseased peas from the United States yielded two apparently new streak-producing viruses—the MS pea streak virus from Minnesota and the PO virus from New York. Another virus isolate, designated the Idaho strain, gave evidence of being a new strain of the Wisconsin pea streak virus. The PO virus caused a peculiar apical necrosis of diseased peas. It was the only virus studied not limited in infectivity to leguminous plants, except that the Wisconsin pea streak virus infected tomato. All 3 viruses were highly infective on all 16 pea varieties tested, but none of the 14 bean varieties studied was susceptible. The Idaho isolate was inactivated after dilution of 1:10,000,000, heating at 78°C for 10 minutes, and aging *in vitro* for 7 days at room temperature. The MS pea streak virus was not inactivated until it was diluted 1:10,000,000, heated to 64°C, and aged 4 days. The PO virus was inactivated at a dilution of 1:1,000,000, at 66°C, and after aging 4 days. Peas inoculated with any of the viruses, developed symptoms more slowly at lower temperatures (16° and 20°C) than at higher temperatures (24° and 28°C). All 3 viruses were transmitted by the pea aphid (*Macrosiphum pisi* Kalt.). Purified virus preparations were highly infective and absorbed the maximum ultraviolet light at a wave length of about 260 mμ. Electron micrographs showed that the particles associated with the Idaho strain consisted of rods with modal size of about 683×14 mμ. MS pea streak virus rods had a predominant size of 660×14 mμ. The particles of the PO virus appeared to be spherical with a modal diameter measuring about 28 mμ. Antisera prepared from Wisconsin pea streak virus and the Idaho strain reacted positively with their homologous and heterologous viruses, showing their close serological relationship. The antiserum obtained from the MS pea streak virus did not react with either of the former viruses although it did react homologously. The titers of the Wisconsin pea streak virus and the MS pea streak virus were determined to be over 1/2000, and that of Idaho strain over 1/500.

Linford (8) first noted the streak disease of pea (*Pisum sativum* L.) in the United States, in 1928. Similar diseases have since been found in several other parts of the world, and a number of viruses or virus strains have been reported that are capable of causing streak symptoms in pea. Some examples of such viruses are pea streak virus 1 (27), *Pisum* virus 3 (1), Wisconsin pea streak virus (3), alfalfa mosaic virus (27), and cucumber mosaic virus (26, 28). Though these reports have served to indicate which viruses were capable of inciting pea streak symptoms in limited geographical areas, no comprehensive study of the causal viruses of pea streak diseases in the United States has been reported. The study reported here was undertaken to provide more proper identification of the viruses that cause pea diseases with streak symptoms. Preliminary reports have been made (6, 7).

METHODS AND MATERIALS.—A collection of 47 pea plants showing streak symptoms was obtained from many important pea-growing areas from New York to the state of Washington. Expressed sap from these diseased specimens was rubbed on peas, beans, and other differential hosts. It was found that sap from about 20% of the 47 plants did not produce visible symptoms on any of the inoculated hosts, presumably because the viruses were not mechanically transmissible, or were extremely unstable and did not survive even a short period of freezing and storage. One or more viruses were readily obtained from the remaining 80%. The most frequently associated virus was bean virus 2. The recovered isolates were placed in 5 groups on the basis of differential host reaction and symptomatology. Of these isolates, 45% resembled WPSV (Wisconsin pea streak virus), about 37% AMV (alfalfa mosaic virus), and 10% CMV14 (cucumber mosaic virus strain 14); about 8% were not assignable.

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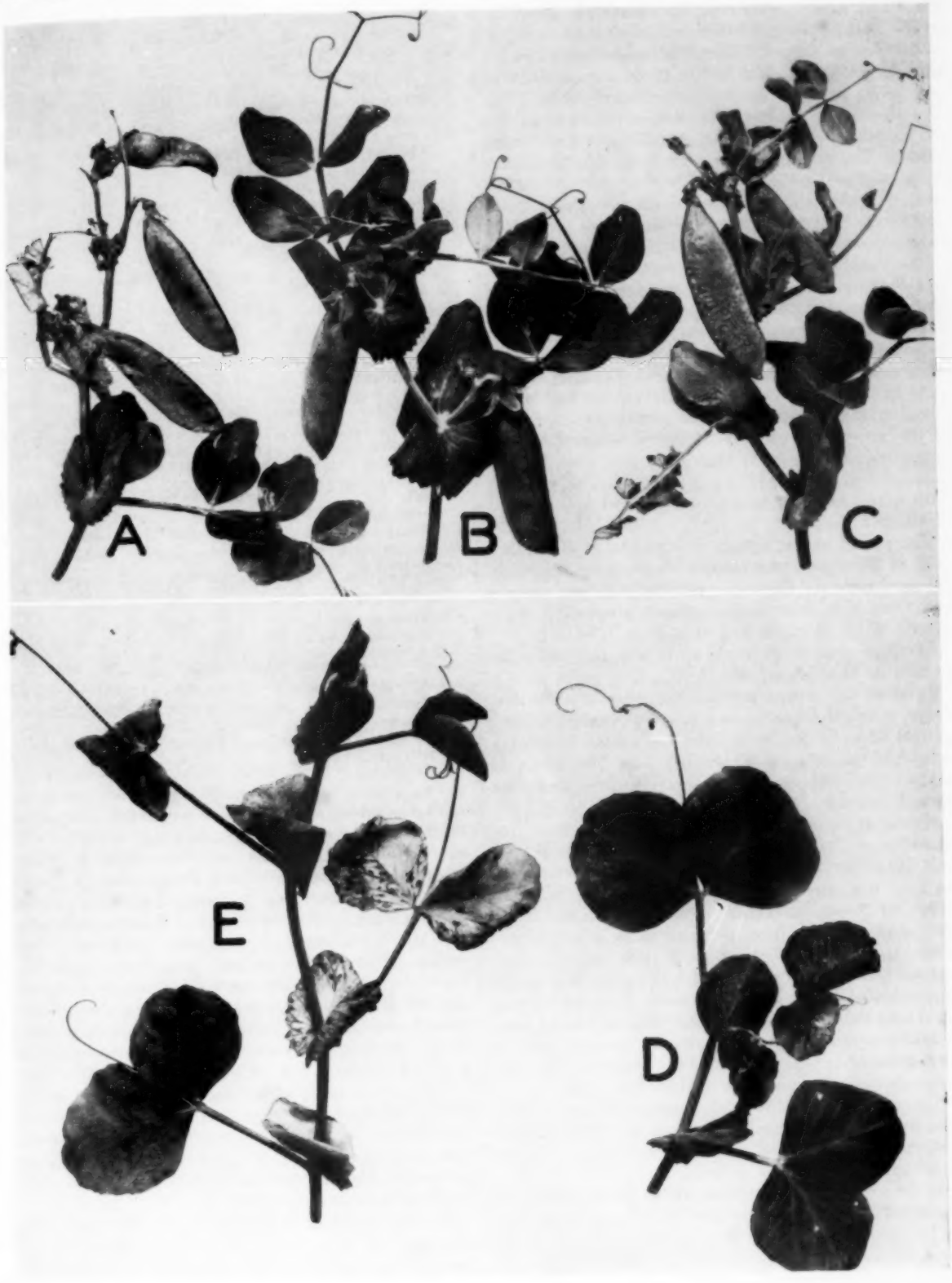
Fig. 1. Symptoms of pea streak on Pride-type peas in the field (A, C) and Perfected Wales in the greenhouse (D, E). The upper portion of a healthy, field-grown Pride-type plant is shown in B. Note similarity of apical foliage chlorosis and necrosis incited by the Wisconsin pea streak virus (A) and the Idaho isolate (C). Also, note foliage malformation and severe veinal necrosis caused by the MS pea streak virus (D) and the severe growing-point necrosis of PO-virus-infected plants (E).

appearing to be different and unique. Two isolates from this "unknown" group and 1 isolate from each of the other 3 groups were chosen at random for detailed study in comparison with 3 known controls—



WPSV, AMV, and CMV14. This paper presents the results obtained when 3 of these new virus isolates (MS and PO from the "unknown" group and I-5 from the WPSV group) were compared with WPSV.

The Idaho isolate, I-5, was obtained from a streak-diseased plant collected near Rigby, in southern Idaho, in 1954. The MS virus isolate came from a streaked pea plant growing in southern Minnesota in 1955. The third virus isolate, PO, was obtained from Dr. W. T. Schroeder, New York Agricultural Experiment Station, Geneva, New York. He had isolated it from streak-diseased peas growing in that state. Our results with this PO virus isolate are included here to provide at least initial comparisons of this interesting virus with WPSV.

The control virus, WPSV, described by Hagedorn and Walker (3), had been maintained in desiccated plant material at the University of Wisconsin.

For the most part, plants to be tested were grown in composted soil in 6-in. earthenware pots. Plants were inoculated mechanically with a glass spatula after carborundum had been sprinkled on the leaves. The Perfected Wales pea variety was used for most of the greenhouse studies. Physical properties were determined in a manner similar to that described by Johnson and Grant (5), except that tests of aging *in vitro* were conducted at a temperature of 22°C instead of 80–90°F.

The virus-free pea aphids (*Macrosiphum pisi* Kalt.) used in the insect-transmission studies were originally obtained from the University of Wisconsin pea nursery and were manipulated by methods similar to those described by Skotland and Hagedorn (19).

Serology tests were made after the technique described by Matthews (10).

In all of the studies undertaken, especially the host range, physical-property, and insect-transmission tests, critical observations were made to ensure that each individual virus studied actually was but a single strain. No evidence of mixtures (variance from the normal reaction) was obtained.

EXPERIMENTAL RESULTS.—*Symptoms on pea.*—Inoculation of Pride-type peas in the field with WPSV first killed the inoculated leaflets and produced chlorosis on the upper foliage (Fig. 1-A). A systemic stem and petiole streaking developed from the basal part of the stem to distal portions of the plant. Meanwhile, the upper foliage showed faint vein-clearing, and the leaflets were malformed and wilted, and finally dropped off. Lower foliage, however, remained green for a long time. Pods rarely filled with seeds, and fine, brownish necrotic spots appeared on the pods, giving them a pitted appearance. Infected plants finally died although control plants were healthy (Fig. 1-B).

The symptoms of the Idaho isolate on pea in the field appeared very much like those of WPSV except that the latter produced somewhat more severe symptoms than the former (Fig. 1-C).

In the greenhouse, symptoms of the Idaho isolate on Perfected Wales peas were slow to develop, requiring an incubation period at 20°C of 14 days. Initial symptoms were wilting and death of inoculated leaflets. A gradual upward wilting of uninoculated foliage followed. Unlike symptoms in the field, the apical foliage did not wilt but remained green as the plant kept growing, and never became severely stunted. A systemic streak developed from the basal part of the stem upward to the distal ends. Faint vein-clearing and slight malformation was often visible on upper foliage. Stem streak became more pronounced with time, and the color changed from steel-gray to copper-brown. Dark-brown oblong or irregular blotches appeared scattered over the older portions of a streaked stem. Infected plants remained alive for a long time, but they seldom recovered to make normal growth.

The streaking symptom was less significant than wilting when WPSV was inoculated on pea in the greenhouse, but the characteristic node-browning was almost always visible. Infected plants were considerably stunted and rarely produced the irregular blotches on the older portions of the stem.

The characteristic symptom of the MS virus isolate on peas was an extensive necrotic streaking on stem and petioles, and an especially severe necrosis of veins

TABLE 1.—*Host range of 3 pea streak viruses in comparison with the Wisconsin pea streak virus* [a]

Host	WPSV	I-5	MS	PO
Cicer arietinum L.	0/6[b]	0/6	10/16	10/10
Crotalaria spectabilis Roth	25/32	22/22	0/16	16/16
Lathyrus hirsutus L.	6/6	0/6	0/6	10/10
Lathyrus odoratus L.	0/10	0/10	10/10	10/10
Lens esculenta Moench	0/10	10/10	0/10	10/10
Lespedeza striata (Thunb.) H. & K.	0/12	0/12	0/12	—
Lupinus albus L.	0/7	0/7	0/7	7/7
Medicago sativa L.	0/15	14/15	15/15	0/8
Melilotus alba Desr.	80/83	23/23	23/23	0/32
Phaseolus aureus Roxb.	0/27	0/27	0/29	0/30
Phaseolus lunatus L.	0/8	0/10	0/17	0/11
Phaseolus vulgaris L.	0/24	0/23	0/26	0/24
Pisum sativum L.	87/100	92/100	91/100	90/100
Pisum sativum var. *arvense* Poir.	10/10	0/40	14/50	10/10
Soja max (L.) Piper	25/33	0/12	0/27	0/26
Trifolium hybridum L.	17/22	L/28[c]	18/28	18/18
Trifolium incarnatum L.	13/15	18/21	L/23	17/20
Trifolium pratense L.	4/22	17/28	L/30	0/22
Trifolium repens L.	3/29	0/34	20/34	0/25
Vicia atropurpurea Desr.	15/19	0/6	0/6	0/6
Vicia faba L.	12/12	17/17	0/16	10/14
Vicia sativa L.	12/12	12/24	0/36	6/48
Vicia villosa Roth	6/11	0/40	4/12	3/13
Vigna sinensis (L.) Endl.	0/28	0/35	10/48	10/24
Cucumis sativus L.	0/21	0/24	0/24	0/15
Capsicum annuum L.	0/6	0/6	0/6	1/16
Datura stramonium L.	0/14	0/15	0/14	6/7
Lycopersicum esculentum Mill.	7/32	0/32	0/32	L/20
Nicotiana glutinosa L.	0/13	0/15	0/13	9/12
Nicotiana rustica L.	0/8	0/8	0/8	6/18
Nicotiana tabacum L.	0/26	0/26	0/28	4/22
Zinnia elegans Jacq.	0/5	0/9	0/8	17/18

[a] WPSV = Wisconsin pea streak virus. I-5 = virus isolate from So. Idaho. MS = virus isolate from Minnesota. PO = virus from New York.
[b] Number of plants infected/number of plants inoculated.
[c] L = virus latent.

TABLE 2.—*Varietal reaction of 16 pea varieties to 3 pea-streak-inciting viruses and the Wisconsin pea streak virus* [a]

Pea variety	% of infection of 20 plants inoculated with			
	WPSV	I-5	MS	PO
Alaska	100	100	50	100
Alsweet	100	100	75	100
Dark-skinned Perfection	100	100	10	100
Early Perfection	100	100	10	100
Horal	100	50	25	65
Large-seeded Perfection	100	50	50	65
New Era	100	50	30	75
New Season	100	100	45	100
New Wales	100	100	30	100
Perfected Wales	100	100	55	95
Pride	100	100	85	65
Resistant Surprise	100	100	45	100
Superior	100	100	25	95
Thomas Laxton	100	100	100	90
Wando	100	100	45	90
Wisconsin Perfection	100	100	45	100

[a] WPSV = Wisconsin pea streak virus. I-5 = virus isolate from So. Idaho. MS = virus isolate from Minnesota. PO = virus from New York.

and veinlets (Fig. 1-D). Symptoms in the field and greenhouse were very similar. The incubation period at 22°C was 10–14 days. Infection was first seen as a curling-back of leaflets at the petiole-end. Meanwhile, severe stem streak developed, extending into the upper foliage. The entire foliage turned chlorotic and was malformed, and the plant died prematurely. When present, pods were discolored and showed necrotic pits on the surface.

The PO virus was unique in its unmistakable incitation of a blight of the growing tip on peas and certain other leguminous plants. In the field, infected Pride-type pea plants, in addition to this top necrosis, developed a systemic brown-purple streaking on stem and petioles. They died prematurely. In the greenhouse, diseased Perfected Wales pea plants rarely showed severe stem streak symptoms, and, except for the tip-blight, the remaining parts of stem and foliage appeared quite normal, although they carried the virus. Leaflets near the blighted apex occasionally developed faint chlorotic spots, especially when the temperature was low. Because of the tip-blight, infected plants could not grow, but they remained alive for some time, until they eventually lost vigor as the dieback progressed down the stem (Fig. 1-E).

Host range.—Thirty-two members of the Leguminosae, Compositae, Cucurbitaceae, and Solanaceae were tested for susceptibility to the four viruses. The plants tested and the results obtained are given in Table 1.

None of the 8 non-leguminous plants were susceptible to the Idaho virus isolate or the MS pea streak virus, but tomato (*Lycopersicum esculentum* Mill.) was infected by WPSV. Fourteen of 24 legumes were susceptible to the WPSV, 10 to I-5 and 12 to the MS streak virus; and 14 of 23 legumes to the PO virus. In addition, the PO virus was infectious to 7 of 8 non-leguminous species tested, and in these tests was latent in tomato. Two legumes, Bountiful bean (*Phaseolus vulgaris* L.) and Chippewa soybean, (*Soja max* (L.) Piper) showed very faint local-lesion-like symptoms after inoculation with the PO virus, but the virus was not recovered therefrom.

Some characteristic symptoms on other hosts.—The Idaho isolate caused marked stunting of diseased lentil (*Lens esculenta* L.) and alfalfa (*Medicago sativa* L.) plants. Leaves of such plants showed a systemic mottle, characterized in alfalfa by the formation of irregularly scattered small chlorotic rings.

When chickpea (*Cicer arietinum* L.) plants were inoculated with the MS virus a systemic streak developed, with wilting and death following in a few days. Symptoms were similar on hairy vetch (*Vicia villosa* Roth.) plants inoculated with this virus, and a vein-clearing of leaves was also observed, soon after introduction of the virus into this host.

Varietal reaction in pea and bean.—The varietal reaction of 16 pea varieties to the four viruses was studied in the greenhouse. Since the results were similar in 2 separate trials, the data are combined in Table 2.

The control virus, WPSV, infected 100% of the

TABLE 3.—*Physical properties of the Idaho virus isolate, the MS pea streak virus, and the PO virus in comparison with the Wisconsin pea streak virus*

Property studied	WPSV	I-5	MS	PO
Tolerance to dilution:				
0	30[a]	30	30	30
10^{-2}	30	28	29	30
10^{-3}	21	11	23	21
10^{-4}	13	6	8	5
10^{-5}	2	6	10	2
10^{-6}	4	4	6	0
10^{-7}	0	0	0	0
Thermal inactivation:				
control	30[a]	30	30	30
56 C	30	30	29	27
58	28	30	25	21
60	28	30	26	15
62	28	30	4	13
64	28	27	0	1
66	25	29	0	0
68	15	24	0	0
70	6	6	0	0
72	11	16	0	0
74	11	13	0	0
76	8	6	0	0
78	2	0	0	0
80	0	0	0	0
Aging *in vitro:*				
0 day	20[b]	20	20	20
1	20	20	20	20
2	16	2	20	9
4	1	2	0	0
7	2	0	0	0
10	6	0	0	0
20	1	0	0	0
30	1	0	0	0
40	1	0	0	0
50	1	0	0	0
60	0	0	0	0

[a] Number of plants diseased out of 30 plants inoculated in 3 trials.

[b] Number of plants diseased out of 20 plants inoculated in 2 trials.

plants inoculated among all varieties tested. The Idaho isolate was also highly infectious on all varieties, but there were small differences in the degree of susceptibility. The MS pea streak virus was least infectious (average 45%) to pea varieties, although it did attack all of them and produced typical symptoms. All pea varieties tested were highly, but not uniformly, susceptible to the PO virus.

The reaction of 14 bean varieties to the 4 viruses was tested in 2 separate trials. The varieties studied included Bountiful, Dwarf Horticultural, Full Measure, Giant Stringless Green Pod, Idaho Refugee, Improved Golden Wax, Kentucky Wonder, Michelite, Pencil-pod Black Wax, Plentiful, Stringless Black Valentine, Stringless Blue Lake, Tender Green, and Tender Pod. None of the varieties developed disease symptoms, nor could any of the 4 viruses be recovered from inoculated plants.

Physical properties.—Tolerance to dilution, thermal inactivation, and resistance to aging *in vitro* results are shown in Table 3.

The WPSV control, I-5, and MS were all finally inactivated at a dilution of 10^{-7} (1:10,000,000), whereas PO was inactivated at 10^{-6} (1:1,000,000). In 3 thermal inactivation tests—heating 10 minutes at 56–80°C. at intervals of 2°C—it was found that WPSV was inactivated at 80°C, I-5 at 78°C, MS at 64°C, and PO at 66°C. Two trials were made of resistance to aging *in vitro*. WPSV was still active at 50 days, but was inactivated at 60 days. I-5 was inactivated in 7 days' aging, and MS and PO in 4 days.

Relation of temperature to disease development.—The effect of temperature on disease development and symptom expression in peas inoculated with the 4 viruses was studied in the greenhouse in 2 separate trials.

Peas were grown at about 20°C. inoculated, and held 4 weeks at 16, 20, 24, and 28°C.

Plants inoculated with WPSV showed early typical symptoms of gray-tan stem discoloration on growing 7 days at 20°C. By this time infected plants at 24°C were beginning to wilt, and at 28°C were dying. At 16°C, symptoms were typical in 10 days.

I-5 incited typical symptoms in 14 days at 20°C. At 24° and 28°C, respectively, diseased plants were showing early wilt symptoms and dying from severe wilting at 7 days. At 16°C it took 28 days for I-5 to produce typical symptoms.

In 7 days, pea plants inoculated with MS showed only typical initial symptoms at 24°C. and were dead at 28°C. Most typical symptoms were seen 14 days after inoculation at 16°C, while at 20°C plants were already wilting after this incubation period.

The PO virus incited earliest typical symptoms after 7 days at 24°C, but fully typical signs did not appear at 16° or at 20°C until after 14 days. At the two higher temperatures, all plants were dead in 14 days, first wilting having been apparent as early as 7 days at 28°C.

Insect transmission.—The pea aphid (*Macrosiphum pisi* Kalt.) was used as the test insect to study the insect-transmissibility of the viruses from pea to pea. The results are summarized in Table 4.

With WPSV not a single infection was obtained in all the transmission series, whereas I-5 and PO showed a high degree of transmission. MS showed maximum infectivity (12%) at the acquisition feeding period of 24-30 hours.

Ultraviolet light absorption tests.—Partial purification of the viruses was performed by processing the sap of infected pea tissue in 0.01*M* sodium phosphate buffer solutions with pH 7.0 and centrifuging at several cycles of low and high speed in a Spinco ultracentrifuge. The purified virus was divided into 3 aliquots—one to test ultraviolet light absorption, one for studying electron microscopy, and one for infectivity tests. The Beckman DU model spectrophotometer was used to determine the ultraviolet light absorption of the preparations. When these partially purified viruses were tested for infectivity on healthy peas, the dilution end points were similar to those shown in Table 3 for unpurified sap.

Table 5 presents the results obtained when optical density studies were made by determining ultraviolet light absorption of diluted preparations of the partially purified viruses. I-5, MS, and PO all showed absorption peaks at the 262-mμ wave length, whereas the peak for WPSV was at 260 mμ.

These results indicate the presence of at least moderate amounts of virus in the diseased or infective sap preparations, and also suggest that the viruses contain fair amounts of nucleic acid.

Electron microscopy.—For electron microscopy, partially purified viruses were mounted on 200-mesh wire grids that had been previously covered with a collodion membrane. Grids were shadowcast with uranium and examined under an RCA electron microscope. Healthy control pea tissue was processed in exactly the same manner as diseased tissue. With these preparations, the morphology of the associated particles was successfully demonstrated under the electron microscope. The figures to follow, representing the sizes of virus particles, are the means calculated from 100 measurements of random virus particles. The particles of WPSV appeared heterogeneous, varying from short rods to long, flexuous fibers and showing a tendency for end-to-end union (20). The modal size was about 14×531 mμ (Fig. 2-B).

I-5 appeared under the electron microscope as rods of more uniform length, predominantly 14×683 mμ

TABLE 4.—*Pea-aphid-transmission tests with the Wisconsin pea streak virus, the Idaho virus isolate, the MS pea streak virus, and the PO virus*

Acquisition feeding period	Per cent of infection[a]			
	WPSV	I-5	MS	PO
15 sec.–1 min.	0	58	2	12
1 min.–30 min.	0	84	2	50
30 min.–60 min.	0	42	10	54
60 min.–90 min.	0	62	0	32
24 hr.–30 hr.	0	42	12	80

[a] Per cent of plants infected among 50 treated with aphids, 5 per plant, that were allowed to feed for 24 hours.

TABLE 5.—*Ultra-violet light absorption determinations of 3 streak-inciting viruses of pea in comparison with the Wisconsin pea streak virus* [a]

Wave length	Optical density [b]			
(mμ)	WPSV	I-5	MS	PO
230	.400	.265	.223	.464
240	.165	.165	.099	.291
245	.154*[c]	.152*	.083*	.260
250	.159	.163	.087	.228*
252	.162	.166	.090	.230
254	.164	.172	.096	.232
256	.165	.176	.095	.234
258	.166	.182	.098	.236
260	.167**[c]	.184	.099	.240
262	.165	.187**	.100**	.242**
264	.163	.186	.099	.227
266	.162	.185	.098	.225
268	.160	.182	.096	.221
270	.150	.179	.095	.216
280	.126	.138	.077	.188
290	.084	.070	.040	.151
300	.044	.040	.020	.116

[a] WPSV = Wisconsin pea streak virus. I-5 = virus isolate from So. Idaho. MS = virus isolate from Minnesota. PO = virus from New York.

[b] Difference in optical density readings of diseased tissue minus healthy tissue preparations.

[c] *Minimum absorption; **maximum absorption.

(Fig. 2-C).

The particles of MS looked more or less similar to those of I-5; they also were homogeneous rods, with modal size 14×660 mμ (Fig. 2-D).

PO particles were somewhat spherical, predominantly about 28 mμ in diameter (Fig. 2-E).

Healthy pea tissue was processed exactly as was diseased tissue. Rod-shaped particles and relatively large spherical particles were not observed in these preparations. However, small spherical particles were observed measuring about 10 mμ in diameter, which might possibly be normal protein particles (Fig. 2-A). This preparation was not infectious to pea.

Serology.[2]—Antisera of WPSV, I-5, and MS were prepared to test the interrelationship of the viruses. Both slide and tube tests were conducted. WPSV and I-5 reacted positively with the reciprocal and homologous antisera, but not with the antiserum of MS. MS did not react positively with either of the antisera prepared from the other two viruses in any of the dilutions from ¼ to 1/2048, although it reacted strongly with the homologous antiserum series. With homologous antisera the titers of both WPSV and MS were over 1/2000, and that of I-5 over 1/500.

On the basis of these serological tests (and other data) I-5 could well be considered a new strain of WPSV, and MS different from the other two viruses.

Cross-protection tests.—Protective inoculation experiments were conducted to obtain information on the possible relationship of PO to cucumber mosaic virus.

When the PO virus was inoculated on zinnia (*Zinnia elegans* Jacq.) it incited systemic mottle. Price's CMV strain No. 6 has been reported to cause local lesions on the same host plant. When zinnia leaves, which had been inoculated with PO virus and developed a systemic mottle, were cross-inoculated with Price's virus, it was found that the PO virus did not protect against infection by Price's virus and necrotic symptoms developed. Tests on 7 zinnia plants gave similar results, indicating no relationship between these 2 viruses.

DISCUSSION.—The experimental data presented are believed sufficient to characterize the pea-streak-inciting viruses I-5, MS, and PO.

The data obtained in these studies with the WPSV agree quite well with those previously reported by Hagedorn and associates (3, 19, 20). The two main differences were that tomato was here found to be a suscept of the virus, and that the thermal inactivation point obtained in these later studies was unaccountably high. These results have been taken into consideration in the following discussion.

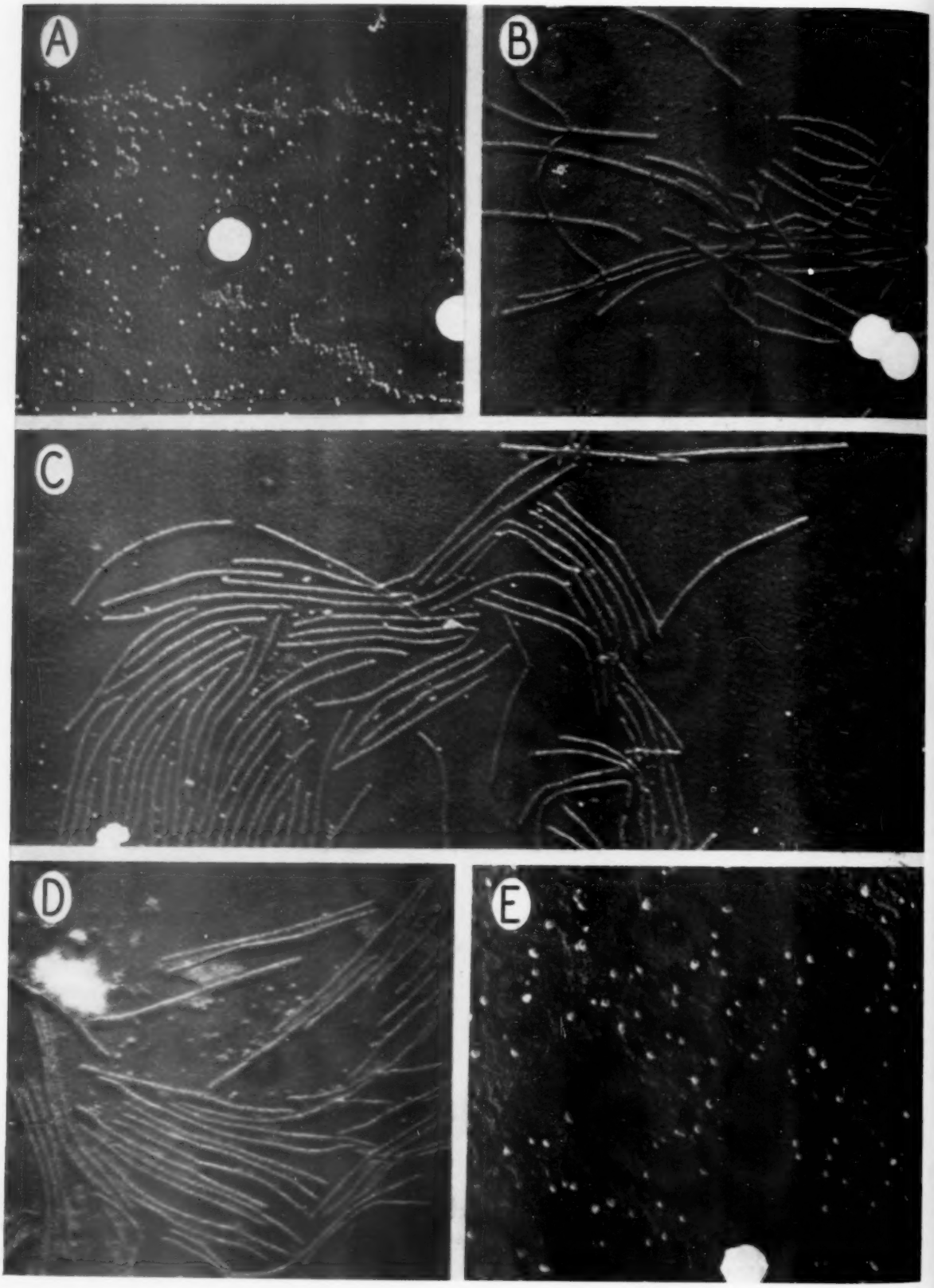
The new Idaho strain of the Wisconsin pea streak virus resembles the original virus described by Hagedorn and Walker (3) in symptoms on peas, physical properties, and rod-shaped particles. The strong reaction of these two viruses in reciprocal antisera is added evidence that they are closely related. However, the Idaho strain differs from WPSV in several ways, the striking differences being aging *in vitro* (the Idaho virus was inactivated within a week while the other lasted as long as 60 days) and pea aphid transmissibility (the Idaho strain was transmitted readily). Some differences were observed in host range among the legumes, but these may well be resolved in further experiments.

The Idaho virus strain can be differentiated from Zaumeyer's pea streak virus 1 (27) by its more stable physical properties and symptoms on pea and other hosts, and from Chamberlain's *Pisum* virus 3 (1) by its ability to infect alfalfa (*Medicago sativa* L.), limited host range in legumes, and transmissibility by pea aphids. The white sweetclover virus (15, 31) cannot be the same virus as the Idaho strain of WPSV, since the former caused mottle on pea, attacked bean (*Phaseolus vulgaris* L.), and was extremely unstable *in vitro*. The Idaho strain is also excluded from the white clover virus or its component viruses, the pea mottle and the pea wilt virus (4, 14, 31) by its inability to attack white clover (*Trifolium repens* L.) and beans, its symptoms on peas, and its higher inactivation points by temperature and dilution. It could also be differentiated from the broad bean local lesion virus (13) and the red clover vein-mosaic virus (2) by its very faint mottle on red clover (*Trifolium pratense* L.), ability to infect alfalfa, and much greater stability *in vitro*.

The new Idaho strain is readily distinguished from AMV and CMV by having a narrower host range, in being more stable *in vitro*, and by the shape and size of the virus particles. None of these previously reported (16, 18) virus particles were long rigid rods.

The Idaho WPSV strain can also be differentiated

[2] Serology was conducted with the kind assistance of Dr. Carl Wetter, Kellogg Foundation Fellow from the Biologische Bundesanstalt, Braunschweig, Germany.


A
B
C
D
E

from other pea-necrosis-inciting viruses like the pineapple yellow-spot virus (9) or tomato spotted-wilt virus (12, 17), tobacco ringspot virus (21, 22), bean red node virus (23), and pea mosaic virus 5 (29) by symptoms on pea, host range, insect transmissibility, and physical properties.

The MS pea streak virus appears to be a new streak virus, for it is different in many ways from the streak-inciting viruses previously described. A few clear-cut contrasting points are emphasized below.

The MS pea streak virus differs from pea streak virus 1 (27) in symptoms on peas, host range, and physical properties. It is different from the *Pisum* virus 3 (1) in greater stability *in vitro*, restricted host range (strictly intra-legume), and transmissibility by the pea aphid. It did not react with antisera prepared with the WPSV virus or its Idaho strain, and *vice versa*.

Since the MS pea streak virus produces no symptoms or only very faint symptoms on the 4 species of clover plants tested, it would be easy to differentiate this virus from clover viruses. Host ranges, symptoms, physical properties, and insect transmissibility of the new virus differ widely from other streak-inciting viruses like tomato spotted-wilt virus (17, 25), tobacco ringspot virus (22), bean red node virus (23), and pea mosaic virus 5 (29).

Because the host range of the pea streak virus is limited to legumes and the particles are long and rod shaped, it can be eliminated from a possible relationship to AMV and CMV.

The new PO virus is unique in symptoms on many legumes. Except in alsike clover (*Trifolium hybridum* L.), crimson clover (*Trifolium incarnatum* L.), and white lupine (*Lupinus albus* L.), it caused primarily a characteristic top necrosis on all the susceptible leguminous plants tested. The virus is clearly differentiated from other streak-causing viruses by this tip-blight symptom, wider host range, ease of pea aphid transmission, and the spherical shape of the virus particles.

The PO virus may well be distinguished from AMV (11, 27, 30) because the latter has a wider host range, produces distinctly different symptoms on legumes and other hosts, and has a different virus particle morphology.

The PO virus particles appear to be spherical. A similar shape has been claimed for strains of CMV (18). However, mottled zinnia leaves infected with the PO virus isolate were not protected against necrotic development upon inculation with Price's No. 6 strain of cucumber mosaic virus, suggesting that the PO virus is not immunologically related to the cucumber mosaic virus.

Similar properties of the PO virus mentioned above may also eliminate the possible relationship of this new virus isolate to clover mosaic and other viruses.

It is significant to note that WPSV, or strains of this virus such as the Idaho strain, appear to be more widespread in geographical distribution than has heretofore been established. The existence of this destructive virus in at least one important pea-growing area of the West may well be noteworthy, especially to those involved in the development of new pea varieties for production there and in the Midwest.

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Fig. 2. Electron micrographs of pea-streak-inciting viruses. **A)** Small, spherical, presumably normal protein particles from healthy pea tissue preparations (×53,000). **B)** Particles of Wisconsin pea streak virus (×53,000). **C)** The more homogeneous rod-shaped particles of the Idaho isolate (×40,000); note similarity to B. **D)** Rod-like particles from MS-diseased pea plants (×40,000). **E)** Roughly spherical particles from PO-infected peas (×65,000). The large, white bodies are polystyrene "balls." Note the presence of tiny, spherical bodies believed to be normal protein particles in all of the electron micrographs.

LITERATURE CITED

1. CHAMBERLAIN, E. E. 1939. Pea streak (Pisum virus 3). N. Z. J. Sci. Tech. **20**: 365A-381A.
2. HAGEDORN, D. J., AND E. W. HANSON. 1951. A comparative study of the viruses causing Wisconsin pea stunt and red clover vein mosaic. Phytopathology **41**: 813-819.
3. HAGEDORN, D. J., AND J. C. WALKER. 1949. Wisconsin pea streak. Phytopathology **39**: 837-847.
4. JOHNSON, F. 1942. The complex nature of white-clover mosaic. Phytopathology **32**: 103-116.
5. JOHNSON, J., AND T. J. GRANT. 1932. The properties of plant viruses from different host species. Phytopathology **22**: 741-757.
6. KIM, W. S. 1958. Studies with several streak-inciting viruses of pea. Ph.D. thesis, University of Wisconsin.
7. KIM, W. S., AND D. J. HAGEDORN. 1957. Studies with virus incitants of pea streak. (Abstr.) Phytopathology **47**: 526.
8. LINFORD, M. B. 1929. Pea diseases in the United States in 1928. Plant Disease Reptr. Suppl. 67, 14 p.
9. LINFORD, M. B. 1932. Transmission of the pineapple yellow-spot virus by Thrips tabaci. Phytopathology **22**: 301-324.
10. MATTHEWS, R. E. F. 1957. Plant virus serology. Cambridge Univ. Press, 128 p.
11. MCWHORTER, F. P. 1949. Alfalfa mosaic virus N. (Abstr.) Phytopathology **39**: 861.
12. PARRIS, G. K. 1940. Mechanical transmission of yellow-spot virus: evidence for identity with spotted-wilt virus. Phytopathology **30**: 299-312.
13. PIERCE, W. H. 1934. Viruses of the bean. Phytopathology **24**: 87-115.
14. PIERCE, W. H. 1935. The identification of certain viruses affecting leguminous plants. J. Agr. Research **51**: 1017-1039.
15. QUANTZ, L., AND J. BRANDES. 1957. Untersuchungen über ein Steinkleevirus. Nachbl. deutsch. Pflanzenschutzdienstes. **9**: 6-10.
16. ROSS, A. F. 1941. Purification and properties of alfalfa-mosaic virus protein. Phytopathology **31**: 394-410.
17. SAKIMURA, K. 1940. Evidence for the identity of the yellow-spot virus with the spotted-wilt virus: experiments with the vector, Thrips tabaci. Phytopathology **30**: 281-299.
18. SILL, W. H., JR., W. C. BURGER, M. A. STAHMANN, AND J. C. WALKER. 1952. Electron microscopy of cucumber virus 1. Phytopathology **42**: 420-422.
19. SKOTLAND, C. B., AND D. J. HAGEDORN. 1954. Aphid transmission of the Wisconsin pea streak virus. Phytopathology **44**: 569-571.
20. STAHMANN, M. A., D. J. HAGEDORN, AND W. C. BURGER.

1950. The electron micrography of the Wisconsin pea-streak virus. Phytopathology **40**: 999-1006.
21. STEERE, R. L. 1953. Tobacco ring-spot virus, a polyhedron, purified with the use of butanol. (Abstr.) Phytopathology **43**: 485.
22. STUBBS, M. W. 1937. Certain viroses of the garden pea, Pisum sativum. Phytopathology **27**: 242-266.
23. THOMAS, H. R., AND W. J. ZAUMEYER. 1950. Red node, a virus disease of beans. Phytopathology **40**: 832-846.
24. WHIPPLE, O. C. 1936. Spotted wilt of garden pea. Phytopathology **26**: 918-920.
25. WHIPPLE, O. C., AND J. C. WALKER. 1941. Strains of cucumber mosaic virus pathogenic on bean and pea. J. Agr. Research **62**: 27-60.
26. ZAUMEYER, W. J. 1938. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. J. Agr. Research **56**: 747-772.
27. ZAUMEYER, W. J. 1939. The new viruses affecting pea (Abstr.) Phytopathology **29**: 25.
28. ZAUMEYER, W. J. 1940. Three previously undescribed mosaic diseases of pea. J. Agr. Research **60**: 433-452.
29. ZAUMEYER, W. J. 1953. Alfalfa yellow mosaic virus systemically infectious to beans. Phytopathology **43**: 38-42.
30. ZAUMEYER, W. J., AND B. L. WADE. 1933. Mosaic diseases affecting different legumes in relation to beans and peas. Phytopathology **23**: 562-564.
31. ZAUMEYER, W. J., AND B. L. WADE. 1936. Pea mosaic and its relation to other legume mosaic viruses. J. Agr. Research **53**: 161-185.

BIOLOGY AND CONTROL OF THE STEM NEMATODE, DITYLENCHUS DIPSACI[1]

K. R. Barker and J. N. Sasser

SUMMARY

Host-range studies were conducted for 2 North Carolina populations of the alfalfa stem nematode, *Ditylenchus dipsaci*. Six plant species and varieties were classified susceptible, 8 slightly susceptible, 10 resistant, and 12 highly resistant. For host differentials, the 2 North Carolina populations from alfalfa, 1 from alfalfa from Nevada, and a Fuller's teasel population from Washington were compared on 9 plant species. The teasel strain did not reproduce on alfalfa, and the alfalfa strains did not reproduce on teasel. The Nevada alfalfa population appeared similar to the Wake County population in that it also reproduced on garden pea. Necrosis appeared in resistant plants, suggesting a hypersensitive reaction. On Wando garden pea, 1 North Carolina population multiplied extensively, whereas the other induced necrosis and did not reproduce. The optimum air temperature for the North Carolina populations of the nematode was 65°F. High moisture favored infection and reproduction. No morphological differences were found in the strains, but the teasel strain was generally larger. An experimental nematocide, *0,0*-diethyl-*0*-2-pyrazinyl phosphorothioate, eliminated the nematode under controlled conditions.

The stem nematode disease of alfalfa, *Medicago sativa* L., is caused by *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (6). The nematode was first described by Kühn (10), on Fuller's teasel, *Dipsacus fullonum* L. Since then, nematodes believed to be *D. dipsaci* have been found infecting about 375 different plant species (4). The pathogen was first observed in the United States by Bessey, in 1907 (11). Allison (1) was the first to observe the nematode infecting alfalfa in North Carolina (Harnett County). Since then, it has been identified on alfalfa in 12 additional counties.

The species *D. dipsaci* includes a number of biological races for which the proper taxonomic status has not been definitely established in all cases (4). Some current investigators maintain that there are no morphological differences among these races (Personal Communication, J. W. Seinhorst), whereas others (12, 17) believe that there are sufficient differences for some of the races to be designated as distinct species. In Europe, certain races are very destructive to alfalfa, rye, oats, peas, red clover, and other crops, and are identified on the basis of the specific host reaction. Races present in the United States attack alfalfa, red clover, rye, onion, teasel, phlox, tulip, narcissus, gladiolus, and many other plants. The disease appears most important on alfalfa and red clover, however. The nematode has been a serious pest on alfalfa in several of the Western States for a number of years, causing severe losses in Nevada and California. Populations of stem nematode on alfalfa have recently been observed in Virginia and North Carolina, where the nematode is posing a serious threat to the production of alfalfa in the Piedmont and Mountain counties.

Measures for controlling the stem nematode depend primarily on proper rotation programs, cultural practices, clean seed, and resistant varieties. Considerable effort has gone into breeding resistant varieties of alfalfa, oats, red clover, and other crops attacked by the pathogen. Unfortunately, the resistant varieties so far developed are not equally resistant to all populations of the nematode. Smith (15) reported that a resistant variety of alfalfa was more resistant to some "biological races" than to others. The alfalfa variety Lahontan was reported highly resistant in Nevada (16), but only moderately resistant in North Carolina (2).

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Several insecticides and nematocides have also been used in recent years in attempts to control the stem nematodes, and success has been obtained in limited experiments (3, 8).

Because of the lack of adequate control measures and the increasing importance of the stem nematode on alfalfa, these investigations were initiated with the following objectives: 1) to determine the host range of 2 North Carolina populations of the nematode; 2) to compare host specificity and morphology of 4 populations of the species; 3) to develop techniques for rapid evaluation of alfalfa breeding lines for resistance; 4) to study the histopathlogical development in resistant and susceptible plants inoculated with 2 populations of the nematode; and 5) to evaluate some chemical methods of control.

MATERIALS AND METHODS.—*Source and maintenance of populations.*—Three populations of the stem nematode from alfalfa and one population from Fuller's teasel were used in these investigations. Two of the alfalfa populations were collected from infested alfalfa fields in North Carolina—one on the N. C. State College Dairy Farm (Wake County) and the other from plots on the Mountain Research Station, Waynesville (Haywood County). The third alfalfa population was obtained from Dr. O. F. Smith, Reno, Nevada. The population from Fuller's teasel was furnished by Mr. W. D. Courtney, Puyallup, Washington. The alfalfa populations from Nevada and Wake County were propagated on Atlantic alfalfa, Wando garden peas, *Pisum sativum* L., and Lee soybean, *Glycine max.* (L.) Merr. The Haywood County population was maintained on Atlantic alfalfa and Lee soybean. The teasel population was increased on Fuller's teasel. All experiments, unless otherwise indicated, were conducted in a controlled temperature room at 65°F. Fluorescent light provided sufficient illumination for plant growth.

Preparation of inoculum.—Infected plants were washed, dried, and stored a month or longer before the nematodes were extracted. The washing and drying reduced the number of a saprophagus nematode, *Panagrolaimus rigidus* (Schn. 1866) Thorne 1937, and certain bacteria that were often present in plants infected with the stem nematode. Seinhorst's extraction technique (7) was modified and used in obtaining large quantities of inoculum. Infected plants were placed on a 100-mesh sieve (8 in. in diameter) supported over an open container. The bottom of the sieve was covered with Scotties facial tissue paper to reduce the amount of debris going through the sieve. A continuous fine mist of water was sprayed on the plants by a cone nozzle (Steinen 1.35, 80°H). Most of the nematodes emerged from the plants within 24–36 hours, passed through the sieve, and settled to the bottom of the container. The continuous fine mist of water resulted in a high rate of recovery of active nematodes.

The extracted nematodes were concentrated by pouring the suspension onto a 325-mesh sieve. Debris was removed by allowing the nematodes to pass through Baermann funnels. The nematodes were drawn from the funnels and filtered out on filter paper (9 cm diameter). The filter papers containing the nematodes were allowed to dry at room temperature, placed in Petri dishes, and stored in a refrigerator at 1–4°C. Under these conditions, adult nematodes died within a few weeks but the larvae remained in a quiescent state for a long time. With these techniques, large quantities of inoculum could be collected and stored for availability at all times. Stored inoculum was prepared for inoculation by placing pieces of filter paper containing the nematodes in a beaker of water. In a short time the nematodes became active and ready for use. The approximate concentration of active nematodes in the suspension was determined by measuring out 2-ml aliquots with a 25-ml pipette and counting the nematodes under a dissecting microscope. The suspensions were diluted for inoculation to a concentration of about 100 active nematodes per ml. When small amounts of inoculum were needed, the Baermann-funnel technique (7) was used for extracting nematodes directly from infected plants.

Inoculation procedures.—The method of inoculation used consisted of direct application of a suspension of nematodes to the seed of the test plants at the time of seeding. In the host range and "strain differential" experiments and in all inoculations after seeding, about 100 active nematodes were used per seed or plant. For all other tests, about 30 nematodes per seed were used. Test plants were grown in 4-in. clay pots filled with soil, with about ⅛–¼ in. of sand over the surface. The soil was steam-sterilized and saturated with water, and the seed was placed about ¼ in. below the surface of the sand. The suspension of nematodes was placed directly on the seed with a medicine dropper, and the seeds were immediately covered with a thin layer of sand. A thin layer of soil was then added to keep the sand moist and to prevent the seeds from being washed out of the sand during watering. The pots were watered daily for 4–5 days to ensure a high per cent of infection.

Assay techniques.—Two techniques were used in assaying plants for infection and nematode reproduction. Young seedlings were examined 2–3 weeks after planting. The nematodes were stained by boiling the plants for 4–5 minutes in cotton-blue–lactophenol (7). After staining and clearing, the plants were crushed between two glass slides, and counts were made under a dissecting microscope. Older plants were examined 5–6 weeks after planting. The tissue was teased into small pieces and placed in Baermann funnels. The nematodes were drawn off about 6 hours after the material was placed in the funnels. The suspensions were poured into transparent plastic dishes, and the number of nematodes determined under a dissecting microscope. Parallel guide lines etched on the bottom of the dishes facilitated counting. Dilutions were made when samples appeared to contain more than 200 nematodes.

Host-range studies.—Thirty-six plant species and varieties were tested to determine their susceptibility to the Wake and Haywood County populations of

D. dipsaci collected from alfalfa. The test plants included important crops grown in North Carolina that might be affected by the nematode, certain weeds that have been reported as hosts, and crop plants that might be used in a rotation program.

Differentiation of populations by host reaction.—Nine plant species, suggested by Seinhorst (14), were used as host differentials. These plants were: teasel, alfalfa, garden peas, red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), Irish potato (*Solanum tuberosum* L.), daffodil (*Narcissus* sp. L.) hyacinth (*Hyacinthus orientalis* L.), and tulip (*Tulipa gesneriana* L.). Alfalfa populations from Wake County and Haywood County, North Carolina, and from Nevada, and the teasel population from Washington, were used as inoculum.

Resistance studies.—The reactions of resistant and susceptible seedlings were studied in 2 susceptible varieties, DuPuits and Atlantic; one resistant variety, Lahontan; a backcross line derived from a cross of DuPuits × Lahontan; and a polycross line selected from uninfected plants growing in an infested field. The backcross and polycross seeds were furnished by Dr. C. H. Hanson, U. S. D. A. Eight-inch clay pots filled with steam-sterilized soil were divided radially into 5 sections. Twelve seeds of a different variety or line were planted in each section. Inoculum used in this experiment, from the Wake County population, consisted solely of larvae. Visual readings were taken 18 days after planting, and the plants were assigned numbers by which they were identified in subsequent assays.

Histological studies.—Infected alfalfa and garden pea seedlings were killed and fixed in formalin–alcohol–acetic-acid solution (9). Plants were selected for fixing 3, 4, 7, 10, 14, and 19 days after planting. The killed and fixed plants were dehydrated with a tertiary butyl alcohol series. The plants were embedded in tissuemat, and sections 20 μ thick were mounted with Haupt's adhesive and 4% formalin. The sections were stained with safranin and fast-green (13).

Chemical control.—The effectiveness of two nematocides, Nemagon (1,2-dibromo-3-chloropropane) and an experimental nematocide, Cole No. 18133 (*0,0*-diethyl-*0*-2-pyrazinyl phosphorothioate), in controlling the stem nematode was investigated. The materials were used in granular form at different rates and with various application-inoculation procedures (Table 3). Inoculum from the Wake Couny population was used in this study. In the first experiment, the nematode inoculum and 3 different dosages of the chemicals (2, 4, and 6 gal./A. of Nemagon and 4, 8, and 16 lb./A. of 18133) were applied at the time of seeding. In a second experiment, the inoculum was applied 1 week after seeding and the chemicals at time of seeding. In a third experiment, both the inoculum and the chemical were applied 1 week after seeding. In the fourth experiment, the inoculum was applied at time of seeding and the chemical 1 week after seeding. In the latter 3 experiments, only the highest rates of the chemicals were used—6 gal./A. of 18133. In experiments 1 and 2, the chemicals were applied at time of seeding and mixed uniformly with the soil mass. In experiments 3 and 4, the chemicals were mixed only with the top inch of soil.

RESULTS.—*Host-range studies.*—Thirty-six plant species and varieties were divided into 4 groups on the basis of infection and reproduction during a period of 6 weeks. These groups were: 1) plants susceptible for nematode reproduction, with an average of over 100 nematodes per plant; 2) plants slightly susceptible, with an average of 100 nematodes or less per plant but with some reproduction occurring; 3) resistant plants, in which infection occurred but no reproduction; and 4) highly resistant plants, in which no infection was observed. Three alfalfa varieties (Atlantic, Buffalo, Du Puits), Wade bean, and Lee soybean were the most susceptible hosts to the Wake and Haywood County populations of the stem nematode (Table 1). Garden pea proved to be a very poor host for the Haywood County population but an excellent host for the Wake County population. This host difference was studied further in the strain and histological investigations. Eight of the plant species and varieties were slightly susceptible hosts to both alfalfa populations. Ten species and varieties were classified as resistant. Twelve plant species appeared to be highly resistant hosts to the alfalfa populations of the stem nematode, for no infection was observed.

Several weed species not previously reported as hosts in North Carolina were infected in infested alfalfa fields: buttercup (*Ranunculus abortivus* L.), speedwell (*Veronica arvensis* L.), neckweed (*Veronica peregrina* L.), dead nettle (*Lamium purpureum* L.), false daisy (*Erigeron anuus* (L.) pers.), chickweed (*Stellaria media* (L.) Cyrill.), dandelion (*Taraxacum officinale* (L.) Weber), and garlic (*Allium vineale* L.). Reproduction was apparent in all these plants except *Veronica arvensis* and *Taraxacum officinale*. All these plants were tested in the controlled-temperature room (65°F) except *Erigeron anuus*, *Ranunculus abortivus*, and the 2 *Veronica* spp., all of which, though planted, failed to grow under the controlled conditions.

Differentiation of populations by host reaction.—Wando garden peas proved to be an ideal differential host for the Wake County and Haywood County populations of the stem nematode. This host supported a large build-up of the Wake County and Nevada populations, but the Haywood County population failed to reproduce on it. When inoculated with the Haywood County population, garden peas reacted in a hypersensitive manner, resulting in necrosis and death of the apical meristem. In consequence, a lateral bud assumed apical dominance and developed into a normal plant. A similar reaction occurred in Atlantic alfalfa plants inoculated with the teasel population. The teasel nematodes infected the alfalfa plants, and typical swellings appeared, but the plants became necrotic in the swollen areas and the nematode failed to reproduce.

The 4 populations were studied and compared for morphological differences. Although the teasel population or race was generally larger in body size, no significant differences were found in other more im-

portant taxonomic characters.

Resistance studies.—The degree of resistance or susceptibility of several varieties and selections of alfalfa was determined in the resistance experiments. The plants were classified on the basis of plant reaction to infection by the nematode. These classes were: 1) plants with slight swellings and necrotic spots; 2) plants with typical swellings and no necrosis; and 3) plants with no apparent symptoms of infection. The early appearance of necrosis was usually limited to the resistant varieties and lines. Large swellings without necrosis were associated with susceptibility. Plants with no symptoms were usually not infected.

The necrotic reaction first appeared in resistant plants around the area where the nematodes were most concentrated, often spreading over the cotyledons and stem. In some cases at 65°F, the reaction was severe enough to kill the plants. Very little necrosis appeared in any of the plants at 75°F. Little reproduction occurred in plants in which necrosis appeared early (Table 2). The backcross of Lahontan × Du Puits had some resistant plants, but the percentage was much lower than in Lahontan. Very little necrosis was apparent in the polycross of the field selections 18 days after planting. Although the rate of infection in the polycross was high, the rate of reproduction was lower than in the susceptible varieties.

A high percentage of the plants in all varieties and lines reacted to infection with typical swellings and no necrosis. In all cases, the rate of reproduction was much greater than in plants of the same variety that exhibited necrosis. At 75°F there was no reproduction in the variety Lahontan, whereas build-up of the nematode populations was considerable in the susceptible varieties. Several plants in all varieties had

TABLE 1.—*Susceptibility of various plant species and varieties to populations of Ditylenchus dipsaci from 2 counties in North Carolina*

Host	Variety	Nematodes per plant [a]	
		Wake Co.	Haywood Co.
Susceptible (average over 100 nematodes per plant)			
Glycine max (L.) Merr., soybean	Lee	408	565
Medicago sativa L., alfalfa	Atlantic	414	267
	Buffalo	244	204
	Du Puits	367	172
Phaseolus vulgaris L., bean	Wade	509	266
Pisum sativum L., garden pea	Wando	802	6 [b]
Slightly susceptible (100 nematodes or fewer per plant, with some reproduction)			
Iris sp. L., iris	Dutch Iris	75	[c]
Lycopersicum esculentum Mill., tomato	Marglobe	34	5
Medicago sativa L., alfalfa	Lahontan	27	28
	Talent	71	92
Nicotiana tabacum L., tobacco (burley)	Kentucky 16	19	11
Pisum sativum L., Austrian winter pea		88	11
Secale cereale L., rye		37	19
Trifolium pratense L., red clover	Kenland	62	40
Resistant (infection observed, but no reproduction)			
Allium cepa L., onion		0	2
Arachis hypogaea L., peanut	N.C. 2	8	14
Cerastium viscosum L., mouse-ear chickweed		0	2
Dipsacus fullonum L., fuller's teasel		0	8
Solanum tuberosum L., potato	Irish Cobbler	19	7
Taraxacum officinale (L.) Weber, dandelion		2	0
Trifolium incarnatum L., crimson clover		0	6
Trifolium repens L., white clover	Ladino	2	5
	White Dutch	3	2
Tulipa gesneriana L., tulip	Advance	3 [d]	7.0
Highly resistant (no infection observed)			
Allium vineale L., garlic [e]		0	0
Avena sativa L., oats	Broncho	0	0
Daucus carota sativa L., carrot		0	0
Festuca elatior L., tall fescue	Kentucky 31	0	0
Hordeum vulgare L., barley		0	0
Hyacinthus orientalis L., hyacinth	Deft's Blue	0	0
Narcissus poeticus L., narcissus	Paper White	0	0
Narcissus sp. L., daffodil	Unsurpassable	0	0
Nicotiana tabacum L., flue-cured tobacco	Hicks	0	0
Poa pratensis L., Kentucky bluegrass		0	0
Stellaria media (L.) Cyrill., chickweed [e]		0	0
Zea mays L., corn		0	0

[a] Mean number of nematodes from six plants.
[b] Not a favorable host for Haywood County population.
[c] Plant not tested with Haywood County population.
[d] Reproduction occurred when nematodes were injected into the bulbs and the plants were grown 3 months.
[e] Infection and reproduction was observed on these plants growing in infested alfalfa fields.

TABLE 2.—*Infection and reproduction of the Wake County population of Ditylenchus dipsaci*

Alfalfa variety	% plants infected	% plants showing necrosis [a]	Nematodes per plant [b]			
			20 days after planting		42 days after planting	
			Swelling	Necrosis	Swelling	Necrosis
Lahontan	78.3	42.0	7.0	5.0	59.0	14.0
Backcross (Lahontan × Du Puits)	84.2	12.9	8.7	0.3	1269.0	0.3
Polycross of field selections	83.3	0	15.0	–[c]	189.0	
Atlantic	88.7	0	13.7	–	1027.0	
Du Puits	87.0	0	11.5	–	732.0	

[a] Swelling is associated with susceptibility, and necrosis with resistance.
[b] Mean of 9 plants with swellings; the number of plants with necrosis varied.
[c] Minus indicates no plants present with respective host reaction.

no apparent symptoms of infection at 65 and 75°F; the percentage of symptomless plants was much greater at 75°F. Assays of these plants made 20 and 42 days after planting showed that most were not infected. When they were infected, the nematodes were in hypocotyl or stem.

Histological studies.—In the histological investigations of resistance, a resistant variety of alfalfa (Lahontan), a susceptible variety (Atlantic), and Wando garden pea were used. Each variety or plant species was inoculated with nematodes from the Wake County and Haywood County populations. Differences in the staining reaction of resistant and susceptible plants of the same variety inoculated with the different populations were studied. Safranin was concentrated in the areas around the nematodes in garden peas if inoculated with the Haywood County population, but not if inoculated with the Wake County population (Fig. 1). The staining reaction was the same in resistant alfalfa plants. This staining difference was first apparent in 7-day-old plants and became more prevalent in older plants. There were no apparent differences in the resistant plants inoculated with the different alfalfa populations of the nematode. The cellular structure of the plants invaded by the nematode was disrupted. The separation of cells was more apparent in susceptible plants (Fig. 1).

Chemical control.—In nematocide experiments, the experimental material No. 18133 gave excellent control of the nematode at all rates used and times of application tested (Table 3). It gave perfect control when applied at the time of seeding, and killed most of the nematodes when applied 1 week after inoculation. Infection was prevented when 1-week-old seedlings were inoculated in treatments in which the chemical was applied at the time of seeding or at the time of inoculation. No reproduction was observed in any of the plants treated with this material. Infection and reproduction was reduced by Nemagon if applied at the time of seeding, but only slightly reduced if applied after inoculation (Table 3). Some infection and reproduction occurred in all treatments in which Nemagon was used.

DISCUSSION.—Information from the host-range studies should prove valuable in planning rotation programs in North Carolina in fields infested with alfalfa populations of the stem nematode. Crop plants such as soybeans and garden peas, found to be highly favorable for development of the nematode, should not be used in rotation with alfalfa. Weed hosts such as dandelion, mouse-ear chickweed, and others should be eliminated. Although resistant varieties are available,

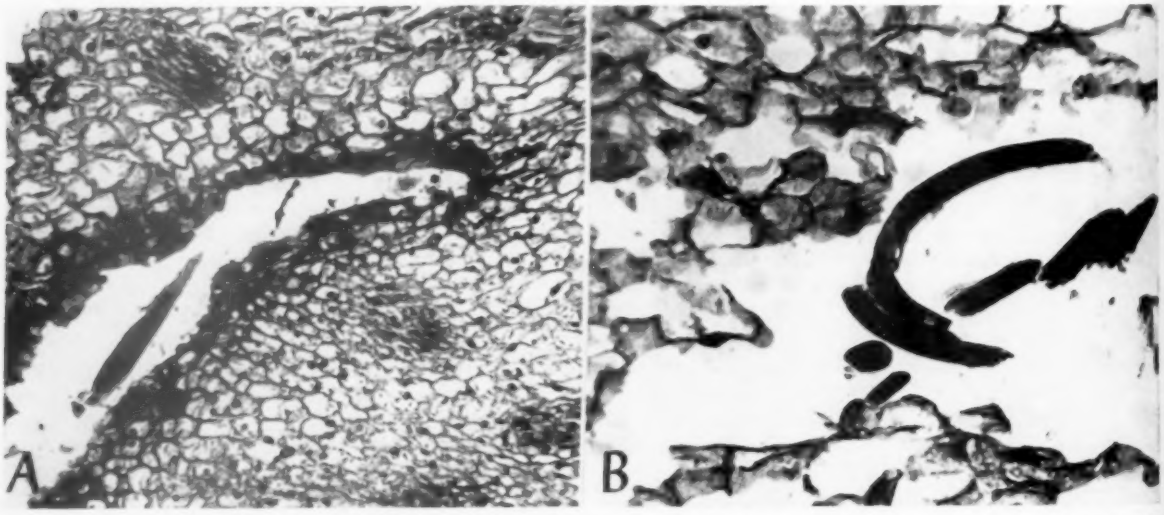

Fig. 1. Photomicrographs of parenchymatous tissue in the zone of the apical meristem of garden peas infected with *D. dipsaci* (19 days after inoculation). **A)** Haywood County population. **B)** Wake County population.

TABLE 3.—*Effects of 2 nematocides in controlling the Wake County population of Ditylenchus dipsaci on alfalfa (variety Atlantic)*

Chemical applied	Rate/A.	Time of application	Time of inoculation	Nematodes per plant [a] 3 weeks after planting	Nematodes per plant [a] 6 weeks after planting
Expt. material 18133	4 lb.	at seeding	at seeding	0	0
Expt. material 18133	8 lb.	at seeding	at seeding	0	0
Expt. material 18133	16 lb.	at seeding	at seeding	0	0
Nemagon	2 gal.	at seeding	at seeding	11.0	85.0
Nemagon	4 gal.	at seeding	at seeding	8.2	16.7
Nemagon	6 gal.	at seeding	at seeding	7.9	50.0
Check			at seeding	14.8	185.3
Expt. material 18133	16 lb.	at seeding	1 week after seeding	0.6	0
Nemagon	6 gal.	at seeding	1 week after seeding	4.8	8.7
Check			1 week after seeding	7.0	21.7
Expt. material 18133	16 lb.	1 week after seeding	1 week after seeding	0	0
Nemagon	6 gal.	1 week after seeding	1 week after seeding	5.6	10.0
Check			1 week after seeding	5.5	20.1
Expt. material 18133	16 lb.	1 week after seeding	at seeding	6.5	0.5
Nemagon	6 gal.	1 week after seeding	at seeding	15.0	91.7
Check			at seeding	15.2	132.1

[a] Mean of 6 plants.

rotation would seem desirable, since some reproduction occurred in resistant varieties. The continued use of resistant varieties might tend to favor the development of more specialized populations of the nematode. The studies on differentiation of populations by host reaction showed striking differences in host specificity between the alfalfa and teasel populations and within the alfalfa populations. The alfalfa populations would not reproduce on Fuller's teasel, and nematodes from teasel failed to reproduce on alfalfa. Garden pea proved an excellent host for differentiating the Wake County and Haywood County populations. These populations apparently have a different ancestral history that could account for the host difference. Differences in the pathogenicity of these 2 populations on resistant varieties might show up under field conditions. The Nevada alfalfa population appeared similar to the Wake County population in that it also reproduced on garden pea, though it appeared less virulent.

Inoculated with the Wake County population, the variety Lahontan did not exhibit the degree of resistance that Smith (16) observed in this variety in tests with Nevada populations of the nematode. The difference in susceptibility might be due to physiological differences in the nematode populations at the 2 locations, or possibly to the different environmental conditions under which the tests were conducted. The same factors may also explain Allison's (2) findings that the same variety was only moderately resistant to the Haywood County population.

The techniques developed for testing the degree of resistance of alfalfa varieties under controlled conditions may assist resistance breeding programs considerably. Plants with the highest degree of resistance developed necrotic spots when infected by the nematode and could be selected and increased in the greenhouse. The necrosis or browning apparently developed as a result of a hypersensitive reaction. Few nematodes were found in the necrotic areas. These nematodes were smaller than those in non-necrotic tissue. After 3–6 weeks, many of the nematodes in the resistant plants had died. The necrosis appeared to be very similar to the reaction described by Dijkstra (5) on resistant red clover seedlings. Susceptible seedlings, including all plants with large swellings and little or no necrosis, could be discarded. This practice would be superior to planting all the seed in field plots and harvesting the seed from all the plants. The plants without symptoms of infection might also be discarded since the majority of these plants would be escapes. The percentage of such plants was about the same in all varieties. Dijkstra (5) and other investigators, in similar breeding and selection programs, considered symptomless plants in red clover to be escapes, and discarded them from their breeding programs.

The histological investigations showed that, before visible necrosis appeared, some type of reaction occurred in the resistant plants that caused the infected areas to stain with safranin. The necrosis or hypersensitive reaction was the result of an interaction between the nematodes and the host plant.

The possibility of chemical control of the stem nematode appeared quite promising. The experimental material, *0,0*-diethyl-*0*-2-pyrazinyl phosphorothioate, gave perfect control when applied at the time of seeding at a rate equivalent to 4 lb. per acre. It killed the nematodes when applied to the soil 1 week after inoculation, indicating that it was systemic in action. Henderson and Williams (8) were able to obtain good control of the stem nematode in field experiments when they used parathion at 5 lb. per acre. Since the experimental nematocide used in this study is also an organic phosphate, it may be effective under field conditions also. The fact that the material is an organic phosphate might limit its use if toxic residues remain in the plants. More information would be needed relative to toxic residues before it could be used on a commercial basis.

LITERATURE CITED

1. ALLISON, J. L. 1950. Pasture and forage legume and grass diseases in North Carolina in 1949. Plant Dis-

ease Reptr. **34**: 205.
2. Allison, J. L. 1956. Stem nematode of perennial forage legumes. (Abstr.) Phytopathology **46**: 6.
3. Bergeson, G. B. 1955. The use of systemic phosphates for control of Ditylenchus dipsaci on alfalfa and daffodils. Plant Disease Reptr. **39**: 705-709.
4. Christie, J. R. 1959. Plant nematodes, their bionomics and control. Florida Univ. Agr. Expt. Stas., Gainesville.
5. Dijkstra, J. 1957. Experiences with the breeding of red clover resistant to the stem eelworm. Euphytica **5**: 298-307.
6. Filipjev, I. N. 1936. On the classification of the Tylenchinae. Proc. Helminthol. Soc. Wash., D. C. **3**: 80-82.
7. Goodey, T. 1951. Laboratory methods for work with plant and soil nematodes. Ministry of Agriculture and Fisheries Tech. Bull. 2. 25 p.
8. Henderson, R. G., and A. S. Williams. 1955. Effect of soil insecticide treatments on alfalfa stem nematode. (Abstr.) Phytopathology **45**: 348.
9. Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill. New York. 523 p.
10. Kuhn, J. 1857. Ueber das Vorkommen von Anguillulen in erkrankten Blütenköpfen von Dipsacus fullonum L. Z. Wiss. Zool. **9**: 129-137.
11. Orton, W. A., and Adeline Ames. 1907. Plant diseases in 1907. U. S. Dept. Agr. Yearbook. 577-589.
12. Ouboter, M. P. de Bruyn. 1930. Tylenchus devastatrix Kühn uit narcis en hyacinth. Tijdschr. Plantenziekten **36**: 125-228.
13. Sass, J. E. 1951. Botanical Microtechniques. Ed. 2. The Iowa State College Press, Ames, Iowa. 228 p.
14. Seinhorst, J. W. 1957. Some aspects of the biology and ecology of stem eelworms. Nematologica **2**: 355-361.
15. Smith, O. F. 1951. Biological races of Ditylenchus dipsaci on alfalfa. Phytopathology **41**: 189-190.
16. Smith, O. F. 1955. Breeding alfalfa for resistance to bacterial wilt and the stem nematode. Nevada Univ. Agr. Expt. Sta. Bull. 188. 15 p.
17. Steiner, G. 1956. The problem of the taxon in the nematode genus Ditylenchus and its agricultural implications. XIV Intern. Congr. Zool. Copenhagen, 1953 Proc. 377-378.

THE ROOT ROT COMPLEX OF VITIS VINIFERA IN CALIFORNIA [1]

Luigi Chiarappa [2]

SUMMARY

More than half of 1131 fungi isolated from grapevine roots and naturally infested soils of California vineyards were pythiaceous. Pathogenicity tests in pasteurized soil revealed that 6 species of *Phytophthora*, 1 of *Pythium*, and 2 unidentified fungi stunted young vines and caused root decay; 2 species of *Phytophthora*, 1 of *Pythium*, 1 of *Rhizoctonia*, and 2 unknown fungi stunted vines without apparent root decay. Sand-culture experiments were best suited for determining pathogenicity levels on grapevines. Two isolates of *Pythium ultimum* varied in virulence on Carignane vines. The effect of nutrition on pathogenicity, and the resistance of the rootstock Othello × Solonis No. 1613 to root-decay organisms, were explored.

Introduction.—A decline of unknown cause has continued for the past several years in several irrigated vineyards in the San Joaquin Valley of California. Typical are circular or elliptical areas of weak vines, often extending over large portions of the vineyard.

Delayed and weak growth characterize the vines known to "run out." The foliage is sparse, often yellowish, and subject to burning in the middle of the summer. Very little or no fruit is produced. Canes do not mature properly at the end of the growing season, and are killed back by early frosts. Always associated with the decline are abundant necrosis of large roots, abnormal branching, and reduction of feeder roots. Most seriously affected are vines growing on their own roots.

The destructive effects of root parasites, such as the grape root aphid and several ecto- and endoparasitic nematodes, have apparently masked the effect of soil fungi on the root system of the grapevine. This is evident from the few reports on the subject. Williams and Hewitt (15) observed that *Pythium olygandrum* Drechs. caused a severe root rot of grapevines grown in solution cultures. *P. de baryanum* Hesse has been reported on grapes in Greece (12), and India (8).

This study was conducted to determine the identity and numbers of pathogens causing root rot of grapevines in California, to assess their pathogenicity under reproducible standard conditions, and to explore the effect of nutrition and rootstock variety on the decay.

Materials and methods.—Various techniques were used to obtain representative samples of pathogenic fungi from naturally infested soils. Isolations were made at various times throughout the growing season from vines of Thompson Seedless, Red Malaga, and Emperor in vineyards of Kern and Tulare counties. Spot isolations also were made in widely scattered vineyards where the "decline" was known to occur. Soil samples collected at various depths around individual vines were mixed and used for growing young rootings in 6-in. pots in the greenhouse in an attempt to reproduce the "decline" under controlled conditions. Small portions of these same soil samples were used to assay for fungus population by the soil dilution

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TABLE 1.—*Frequency of isolation and identity of fungi from the rhizosphere of grapevines grown in naturally infested soil*

Fungi isolated	Number of pure cultures obtained	Per cent frequency
Botrytis	3	0.26
Chetomium	1	0.09
Cylindrocarpon	11	0.97
Dactylium	4	0.35
Fusarium	150	13.26
Mucor	16	1.41
Nigrospora	3	0.26
Papularia	10	0.88
Penicillium	1	0.09
Phytophthora	364	32.18
Pythium	295	26.08
Rhizoctonia	135	11.94
Stemphylium	5	0.44
Trichoderma viridis	9	0.80
Unidentified spp.	124	10.96

technique, or they were placed in apples for the isolation of pythiaceous fungi according to the technique of Campbell (3). Grapevine roots showing evident cankers were plated on water agar, to which, in some cases, the antibiotic novobiocin had been added in the amount recommended by Butler and Hine (2). Freshly collected roots were plated directly in the vineyard. Fungus cultures were purified by single hyphal tips, and the genus was identified by means of the keys of Barnett (1) and Gilman (6). Identification of *Pythium* species was made according to the method and key of Middleton (11).

Pathogenicity potentials for each fungus were progressively established, at first retaining only those fungi that caused damping off or root decay in young seedlings. These fungi were then tested on larger vines in pasteurized soil. In these experiments pathogenicity was evaluated by comparing the growth of inoculated vines with that of controls, and by estimating root decay visually. Fungi showing some degree of pathogenicity were used for field inoculations or in sand-culture experiments. In the latter tests the effect of root-rotting fungi on grapevines was evaluated by rating decay on an arbitrary scale and by measuring the growth reduction of inoculated plants. The net increase in green weight for each individual vine was determined by subtracting the weight of the cutting at planting time from the fresh weight of the vine at the end of the experiment.

Preliminary pathogenicity tests on 4–6-week-old grapevine seedlings of the Tokay variety were conducted as follows: the seedlings were individually transplanted from vermiculite flats into pasteurized soil in 10-oz. paper cups, and a mat of fungus was cut into small pieces and mixed with the soil in each cup at transplanting time. Fungus colonies 7-10 days old growing on PDA (potato-dextrose agar) in standard Petri dishes served as inoculum for young vines growing in pasteurized soil or in sand culture. About the same amount of fungus mycelium was placed in each of 4 holes in the soil or sand around each vine. All the vines were inoculated when they were 25 cm tall. Old Red Malaga vines in the vineyard were inoculated with fungi grown on a medium of autoclaved whole oats in 1-qt. Mason jars. This inoculum was distributed in trenches dug around each vine. Six jars of each organism were used in each inoculation.

EXPERIMENTAL RESULTS.—Table 1 lists the fungi isolated from the rhizosphere of grapevines in various vineyards of California. More than half of 1131 isolates were pythiaceous fungi. Of these, 178 cultures were obtained by highly selective isolation methods (e.g., the apple technique), 47 by the soil dilution technique, and the rest by plating. Of the 254 isolates tested for pathogenicity, only 67 showed some degree of pathogenicity. Most pathogens were fungi of the genera *Pythium*, *Phytophthora*, and *Rhizoctonia*. Further tests with these 67 isolates were made on young rootings of Thompson Seedless, Red Malaga, and Carignane vines in pasteurized soil in 6-in. pots. Four replicates for each organism were used, and the pots were maintained in a lath house for 1 year. Six species of *Phytophthora*, 1 of *Pythium*, and 2 unidentified fungi were found to cause growth reduction and root decay; 2 species of *Phytophthora*, 1 of *Pythium*, 1 of *Rhizoctonia*, and 2 unknown fungi caused growth reduction without apparent root decay.

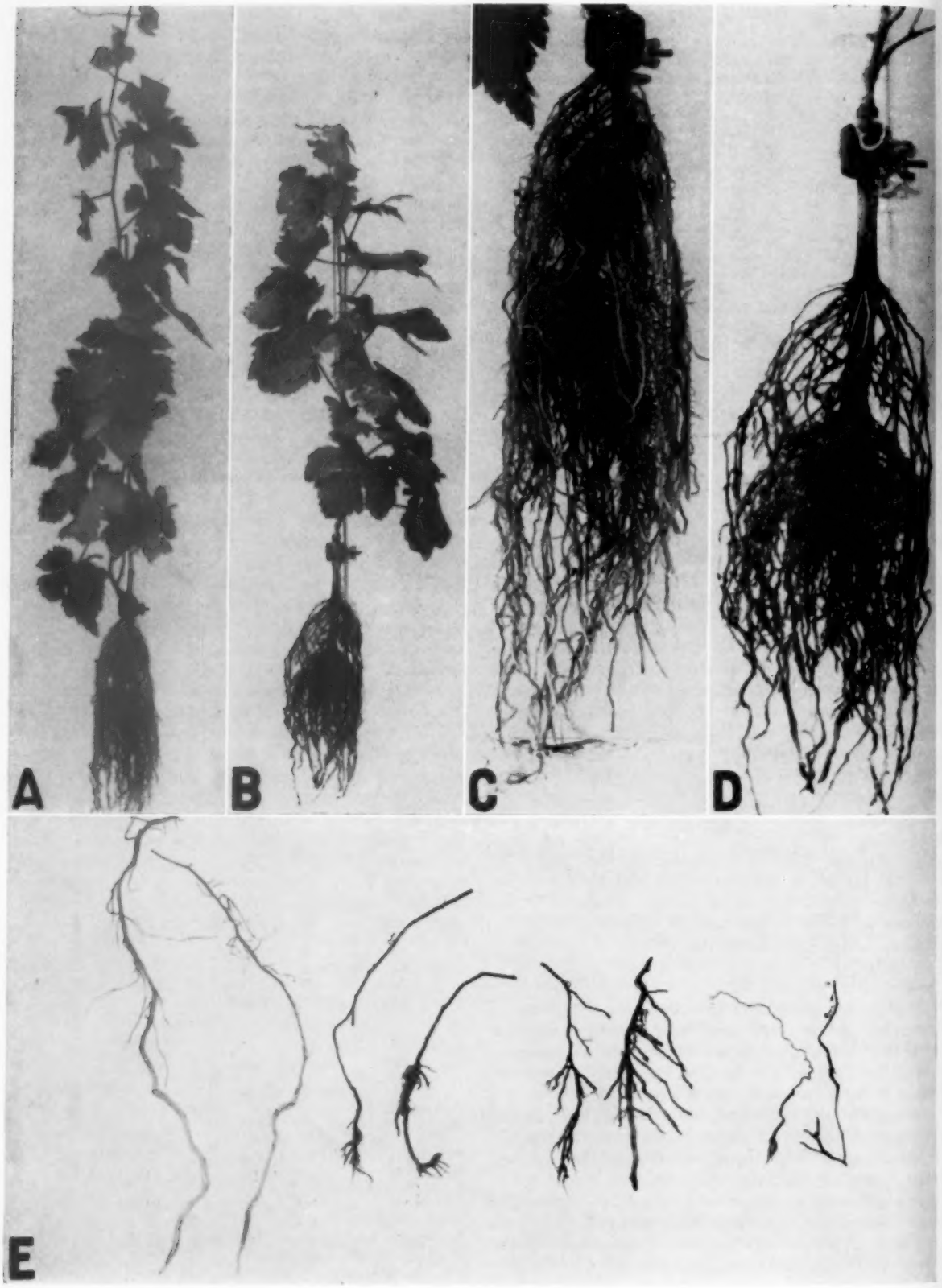
None of the 35 old Red Malaga vines inoculated in the field showed any disease symptoms 13 months after the soil was infested.

Sand-culture experiments were the most useful in establishing the pathogenicity potentials for root-rotting fungi. Table 2 reports the results of an experiment in which Carignane vines were inoculated with various soil organisms and grown 133 days in sand culture at ½ concentration of Hoagland's solution (7). Four vines per crock and 10 replicates per treatment were used. The temperature range was 18–25° C. The vines, inoculated with 2 isolates of *Pythium ultimum* Trow, were stunted (Fig. 1-A,B) and displayed various degrees of leaf chlorosis, somewhat resembling that produced by zinc or iron deficiency (Fig. 2). Attempts to correct for zinc deficiency by a foliar application of chelated zinc sulphate were unsuccessful. In general, growth was more severely impaired by isolate T 3B, and chlorosis was greater with isolate TS 56-1. The root systems of vines inoculated with both these organisms and a *Phytophthora* sp.

TABLE 2.—*Reduction of growth and amount of root decay caused by some soil fungi on young Carignane vines growing in sand culture*

Code number	Identity of inoculum	Average increase in fresh weight (g)	Decay rating*
	Control	74.8	0
P 1	*Rhizoctonia* sp.	65.9	2
RM 42-3	*Rhizoctonia* sp.	71.7	1
E 40-29	*Pythium* sp.	73.1	1
TS 56-1	*P. ultimum*	54.9	3
T 3B	*P. ultimum*	48.0	3
RM 36-5	Unknown	71.3	0
E 48-40	*Phytophthora* sp.	70.3	3

*0 = none; 1 = very mild; 2 = moderate; 3 = severe.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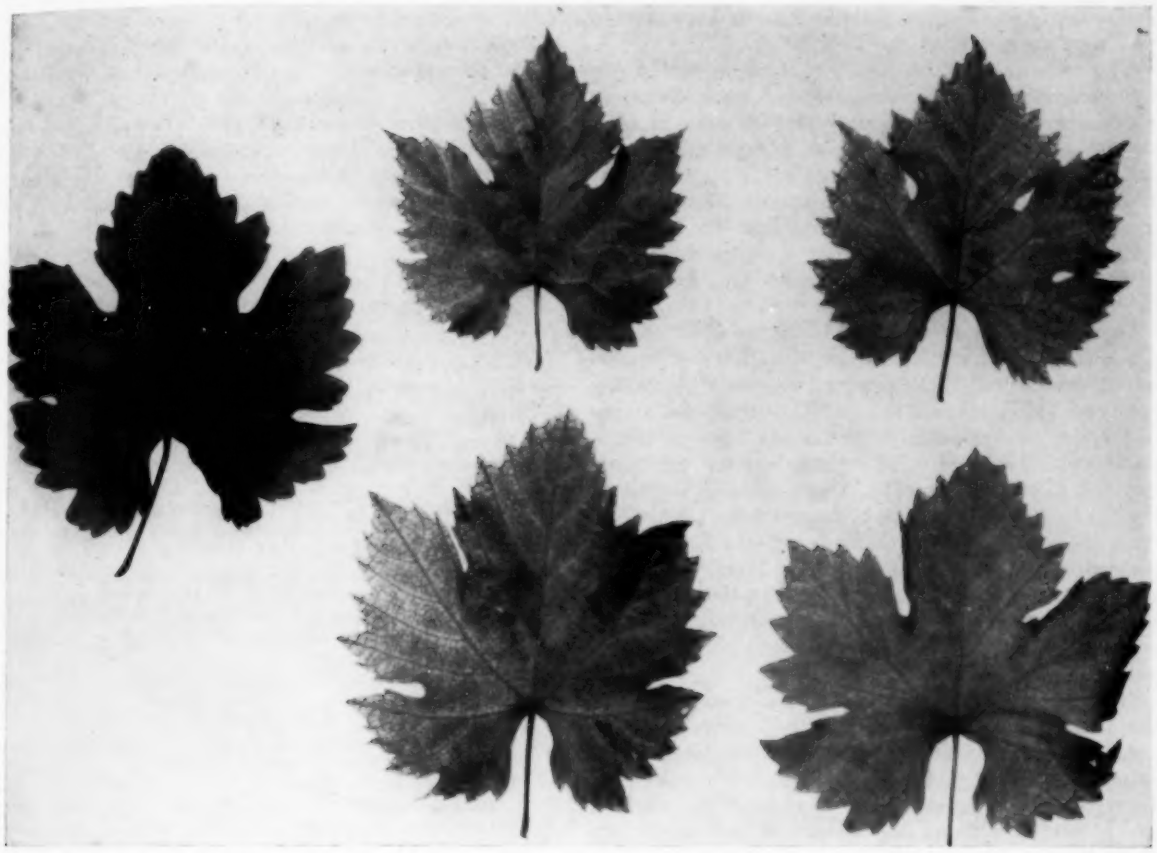

Fig. 2. Chlorosis of Carignane leaves resulting from root decay. Leaf at left is from a control vine; the others are from vines inoculated with *P. ultimum*.

showed an over-all growth inhibition, abundant necrotic lesions, death of root apices, and abnormal production of adventitious rootlets (Fig. 1-C,D,E). Disintegration and sloughing off of the root cortex was one of the prominent symptoms on roots of the vines inoculated with one of the *Rhizoctonia* sp. Reisolations from decayed roots yielded the inoculated organisms.

The effect of nutrition on the pathogenicity of *P. ultimum* (isolate TS 56-1) was tested in a separate sand-culture experiment, in which 3 concentrations of Hoagland's solution (0.125, 1.0, and 1.5) were employed. The Carignane variety and rootstock Othello × Solonis No. 1613 (hereinafter referred to as 1613) were compared in this study for their ability to resist root decay. Two vines per crock and 7 replicates per treatment were used. Temperature in the glasshouse where the plants were growing was 25–32°C. The average net increase in fresh weight of the vines was determined after 56 days of growth. The results of this experiment are summarized in Table 3. From statistical analyses of the data, it appeared that differences in growth from nutrition levels alone were highly significant in both Carignane and 1613 vines, whereas growth reduction due to *Pythium* was significant only in the Carignane vines. Although the mean treatment values for the latter variety appear to indicate some interaction of nutrition and pathogenicity,

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Fig. 1. Stunting and root decay caused by *P. ultimum* on Carignane vines grown in sand culture at ½ Hoagland's solution. **A)** Control vine. **B)** Inoculated vine. **C)** Roots from a control vine. **D)** Roots from an inoculated vine. **E)** Necrosis, small cankers, abnormal branching, and adventitious rootlet formation in roots from inoculated vines. The 2 roots at left are from a control vine.

TABLE 3.—*Effect of nutrition and Pythium ultimum on the growth in sand culture of Carignane vines and 1613 rootstock*

Treatment		Fresh-weight increase (g)	
Strength of Hoagland's solution	Fungus	1613	Carignane
0.125	None	42.1	53.9
0.125	*P. ultimum*	43.9	56.6
1.0	None	92.2	131.6
1.0	*P. ultimum*	98.7	99.8
1.5	None	99.2	114.4
1.5	*P. ultimum*	96.0	79.7
LSD 5%		15.4	24.8
LSD 1%		20.8	33.6

the analysis of variance showed that this interaction was significant only at the 10% level.

The resistance of the 1613 rootstock to root decay was confirmed in a parallel series of experiments, in which these vines were grown for 56 days in sand culture at ½ Hoagland's solution in crocks immersed in a constant-temperature bath at 30±1°C, which corresponds to the optimum for growth of the organisms used as inoculum (*P. ultimum* isolate TS 56-1 and *Rhizoctonia* sp. isolate P 1). No root decay was visible and no significant differences were detected by statistical analyses of the resulting data.

Discussion.—It is recognized that no definite conclusions can be formulated from the survey of fungus populations in the rhizosphere of economic plants, because no sufficiently reliable methods exist for a true qualitative or quantitative evaluation of soil microorganisms. However, the largest number of fungi isolated from the grapevine rhizosphere belonged to the genera *Pythium* and *Phytophthora*. Isolations from different growing regions of California also yielded species of the same genera of fungi. This association (4, 5, 9, 10, 13, 14) suggests that these organisms may be playing a more active part in the root-decay complex of grapevines than has been suspected.

Besides establishing the pathogenicity of several of the isolated fungi, the investigations revealed some points of general interest. The large variability of results obtained in pathogenicity tests with soil-potted vines was eliminated by the sand-culture method, which greatly reduces the effects of soil microflora and unknown factors. It is admitted that a final assay for pathogenicity should be made under "natural" conditions, but the sand-culture technique is believed to offer great advantages in establishing pathogenicity *per se* of soil fungi on woody plants.

The differences in decay and symptomatology caused by 2 isolates of *P. ultimum* obtained from California vineyards seem to indicate that gradations of virulence may exist within the same species. Whether this implies a physiologic specialization of these fungi on grapevines should be put to experimental test.

The growth reduction resulting from the activity of pathogenic soil fungi is highly significant in establishing a probable relationship with the "declining condition" existing in the field.

The effect of nutrition on the pathogenicity of soil organisms appears to be important because of the possibility of practical field applications. It is conceivable, in fact, that appropriate fertilization programs might differentially affect host and parasite, thereby improving growth. Information collected in one of our experiments seems to corroborate this possibility.

The resistance of rootstock 1613 to *P. ultimum*, and its adaptability to the ecological conditions of the central valleys of California, where "declining" vineyards are known to occur, suggests using this rootstock in new replants to control the disease in infested areas.

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LITERATURE CITED

1. Barnett, H. L. 1955. Illustrated genera of imperfect fungi. Burgess Publ. Co., Minneapolis, Minn. 218 p.
2. Butler, E. E., and R. B. Hine. 1958. Use of novobiocin for isolation of fungi from soil. Soil Sci. **85**: 250-254.
3. Campbell, W. A. 1949. A method of isolating Phytophthora cinnamomi directly from soil. Plant Disease Reptr. **33**: 134-135.
4. Crandall, B. S., G. F. Gravatt, and M. M. Ryan. 1945. Root diseases of Castanea species and some coniferous and broad leaf nursery stock caused by Phytophthora cinnamomi. Phytopathology **35**: 162-180.
5. Frazer, Lilian. 1942. Citrus decline in the Murrumbridgee irrigation area: importance of Phytophthora root rot. Agr. Gaz. N. S. Wales **53**: 415-419.
6. Gilman, J. C. 1957. A manual of soil fungi. Iowa State Coll. Press. Ames, Iowa. 450 p.
7. Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347. 32 p.
8. Jain, A. C. 1950. Root rot of grape-vines (Vitis vinifera Linn.) caused by Pythium de baryanum Hesse var. viticulum n. variety. Nagpur Agr. Coll. Magazine **25**: 7-9.
9. Klotz, L. J., and V. P. Sokoloff. 1943. The possible relation of injury and death of small roots to decline and collapse of citrus and avocado. Calif. Citrograph **28**: 86-87.
10. Martin, J. P. 1948. Fungus flora of some California soils in relation to slow decline of citrus trees. Soil Sci. Soc. Am. Proc. **12**: 209-214.
11. Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Torrey Botan. Club Mem. 20. 171 p.
12. Sarejanni, J. A. 1936. Notes phytopathologiques. II. Ann. Inst. Phytopathol. Benaki, Greece. **2**: 86-92.
13. Wager, V. A. 1942. Phytophthora cinnamomi and wet soil in relation to the dying back of avocado trees. Hilgardia **14**: 519-532.
14. Wager, V. A. 1942. Pythiaceous fungi on Citrus. Hilgardia **14**: 535-538.
15. Williams, W. O., and W. B. Hewitt. 1948. Control of grape root-rot in solution culture. Proc. Am. Soc. Hort. Sci. **52**: 279-282.

PHYSIOLOGIC SPECIALIZATION OF PHYTOPHTHORA INFESTANS IN ISRAEL[1]

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SUMMARY

The late blight disease causes severe damage to potato and tomato crops in Israel, with fungicidal control measures frequently insufficient. In 1954–57, to increase knowledge of the physiologic specialization of the pathogen, 48 fungus isolates from blighted potato plants were identified on potato differential hosts, and 25 isolates were tested on tomato differential plants. On commercial potato varieties (genotype rr), race 4 increased from 68% in 1955 to 100% in 1957; race 0 decreased from 24% in 1955 to 0 in 1957. Other races occasionally found were: 1; 3; 1,4; and 1,3,4. The distribution of races over areas ecologically different is not correlated with their pathogenic properties. The tested potato isolates could be subdivided on tomato differential hosts into the following tomato races or subraces; 36%—Berg's original "potato race" (1), innocuous on tomato varieties; 28%—race To, virulent solely on recessive tomato varieties; 16%—T_1, pathogenic on Rutgers and accession W.Va. 106, but avirulent on W.Va. 36-1 and W.Va. 700; 16%—parasitic on Rutgers, W.Va. 106, W.Va. 36-1, and not capable of attacking the accession W.Va. 700; 4%—isolates parasitizing Rutgers and the accessions W.Va. 106, W.Va. 36-1, and W.Va. 700.

The late blight disease caused by *Phytophthora infestans* (Mont.) d By is most destructive to potato and tomato crops in Israel. The principal potato varieties, Up-to-Date and Arran Banner, are cultivated under irrigation in 2 growing seasons. The fall crop, usually planted in late August and harvested in December, originates from seed tubers produced domestically in spring of the same year. Spring potatoes are planted in February-March and harvested in May-June. About 90% of the seed tubers for this more important crop come from abroad, mainly from Scotland and North Ireland. The spring crop yields about 12–16 tons per acre, twice the fall yields.

Potato fields are distributed in most parts of the country over a wide range of climatic conditions, from Galilee, in the north, throughout the Coastal Plain, Valley of Esdraelon, Jordan Valley, and Hills of Judea, reaching the N. Negev in the south. Late blight severely damages both the spring and fall-grown potatoes—on the former primarily showing on the above ground parts, and in the latter appearing on both vines and tubers.

Fungicidal control measures afford satisfactory protection when heavy rains do not interfere with applications, or wash off the protective chemicals. The uncertainty of results and the high cost of materials and labor have in many countries stimulated a search for resistant varieties (5, 19, 20). Similar studies have also been undertaken in Israel. To make such an enterprise successful, a basic knowledge of the physiologic specialization of the pathogen seemed imperative. Information on this problem in the Near East is very scarce; a few isolates collected in Jordan have been identified by European investigators (3, 13, 14).

The main object of the present studies was to identify the physiologic races of *Phytophthora infestans* and to explore the relations between late blight diseases attacking potatoes and tomatoes. Special attention was given to the prevalence and distribution of physiologic races in both growing seasons and in various regions of the country.

IDENTIFICATION OF RACES ON POTATO DIFFERENTIAL VARIETIES.—*Materials and methods.*—Late-blight-affected potato foliage, stems, and tubers were collected from various parts of the country, and incubated 24 hours in high humidity under bell jars or in large Petri dishes. Under these conditions the fungus yielded sporangia profusely. Inoculum was obtained by washing sporangia from the surface of diseased tissue and maintaining suspensions at 12–15°C to stimulate liberation of zoospores. Stock cultures were preserved in tubers artificially inoculated by placing a few drops of spore suspension in small cavities produced in tuber tissue with a knife sterilized by flaming. Inoculated tubers were kept 48 hours at 20–25°C and then removed to incubators at 6–10°C. Every 10 weeks the isolates were transferred to fresh tubers. It was ascertained that the fungus in the tuber cultures retains its racial identity for at least 2 years. The organism could be readily recovered from slices of infected tubers exposed to 16–20°C at high humidity.

In the identification tests the widely used detached-leaf culture method was employed. The bottoms of Petri dishes were lined with moist blotting paper, covered in turn with a thin pad of cotton. Leaflets of 10–12-week-old differential hosts were laid, lower side up, on the cotton.

Inoculations were made by placing 4–6 drops of zoospore suspension on each leaflet. After 12–16 hours the excess of suspension liquid was removed by a small pipette. The inoculated leaves were incubated at 18°C, high humidity being ensured by repeated moistening of the blotting paper. The dependability of the method employed was occasionally checked by running comparative tests on plants in garden pots (11).

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The reliability of the detached-leaf culture technique was thereby confirmed, and was therefore used.

Each identification test was carried out in duplicates or in triplicates and repeated whenever the results of the replications were not consistent. Varietal reactions were observed daily, and the final readings were made 5–7 days after inoculation. Identification of races was based on reactions displayed on the following varieties:

Potato variety	Blight genotype
Irish Cobbler, or Up-to-Date	rr
835a(4)	R_1
1512c(16)	R_2
3RC-8	R_2
Pentland Ace	R_3
1563c(14)	R_4

Since the listed differentials are single gene indicators, purification of races from mixtures was achieved by means of the reinoculation method. For example, inoculum collected from rr variety and virulent on R_1 and R_4 plants, was transferred again from the infested plants of R_1 and R_4 type to healthy R_1 and R_4 leaves. The ability or inability of the fungus to attack both R_1 and R_4 hosts identifies it as race 1,4 in the first instance, or as race 1 or race 4 if only one of the respective differential hosts was attacked.

Host reactions were classified as follows: leaves with necrotic spots of arrested spread were considered resistant; abundant sporulation and unrestricted spread and coalescence of lesions were regarded as evidence of susceptibility (6, 16). Difficulties were experienced when reaction type was not well defined. Leaves supporting very scanty mycelial growth in some replications were rated resistant. When all replications showed sparse mycelial development accompanied by coalescence of necrotic lesions, the host was ranked as susceptible. In such cases, identification tests were repeated, and additional differential varieties sometimes used.

The identity of physiologic races and their nomenclature were determined with the aid of the international system of designating interrelationships of genes and races (4).

Results.—Forty-eight isolates, collected almost exclusively from commercial potato varieties (rr genotype), were tested on potato differential hosts. It was noticed that differentials representing the R_1 genotype, like Essex and 835a(4) are not consistently equivalent in their reactions; some isolates, determined as race 0 or 4, were moderately virulent on Essex, and avirulent on 835a(4) and other varieties of the R_1 category, such as the German Maritta. Similar discrepancies in the response of differential hosts to race 1 had also been observed by Howatt and Grainger (10). Obviously, disagreement in reactions obtained on differentials standing for the same genotype may lead to confusion, and it is therefore suggested to remove Essex from the set of standard differential varieties. The results of the identification studies are recorded in Table 1.

The data summarized in Table 1 demonstrate the prevalence of races 4 and 0 in the development of potato late blight epiphytotics. The same races have been collected occasionally in neighboring Jordan (3, 14). During 1954–57, race 4 accounted for 75%, and race 0 for 15% of the total. Races 1; 3; 1,4; and 1,3,4 make up the balance. Race 4 gradually increased in prominence. In 1954 it was isolated once in 3 samples tested; in 1955 it comprised 68%, in 1956 90%, and in 1957 100% of the isolates obtained from the commercial potato varieties (genotype rr).

The reverse is true of the common race 0. In 1954, race 0 appeared once among 3 isolates studied; in 1955 it amounted to 24% of the total; in 1956 and 1957 not a single isolate of race 0 could be detected. It should be mentioned, however, that race 0 was found on blighted tomatoes in 1956 and 1957. Similar trends and shifts in race populations of *P. infestans* were reported (3) from Europe, North America, and Africa (Southern Rhodesia), where race 0 declined markedly, to be replaced by race 4.

Information accumulated in the present study also indicates that race 4 seems adapted to diverse climatic conditions, since it predominates in fall and winter as well as spring, being widespread over areas strikingly different ecologically and in natural vegetation. Fig. 1 shows the uniform distribution of race 4 in various parts of the country, belonging phytogeographically to the Mediterranean and Irano-Turanian regions (24), while other races are not so evenly distributed.

If the inoculum inciting the recurring late blight outbreaks in Israel is tuber-transmitted, as proved in other countries (5, 18, 23), a correlation may be expected between races parasitizing the parental plants and the progenies of the seed tubers. The similarity in composition of races in fall of 1955 and in spring of the same year when the seed for the fall crop was produced accords with such an anticipation. For the same reason the composition of races parasitizing our potatoes in spring should be influenced by the race situations of the previous year in North Ireland and Scotland, source of 90% of the seed for our spring crop. Comparative analysis of the findings depicted in

Table 1.—*Prevalence of physiologic races of Phytophthora infestans among isolates collected from potatoes in Israel in 1954-57*

Growing season	Source of seed tubers	Frequency of race occurrence[a]			
Fall 1954	Local	0(1)	3(1)	4(1)	
Spring 1955	Local			4(1)	
	Imported	0(3)		4(6)	
	Unknown[b]			4(1)	
Fall 1955	Local	0(3)	1(1)	4(9)	1,4(1)
Spring 1956	Local			4(2)	
	Imported			4(1)	1,4(1)
	Unknown[b]			4(6)	
Spring 1957	Local			4(2)	
	Imported			4(1)	1,3,4(1)
	Unknown[b]			4(6)	

[a] Numbers preceding brackets designate specified races; figures in brackets indicate number of isolates embraced by the race concerned.

[b] 90% of seed tubers imported.

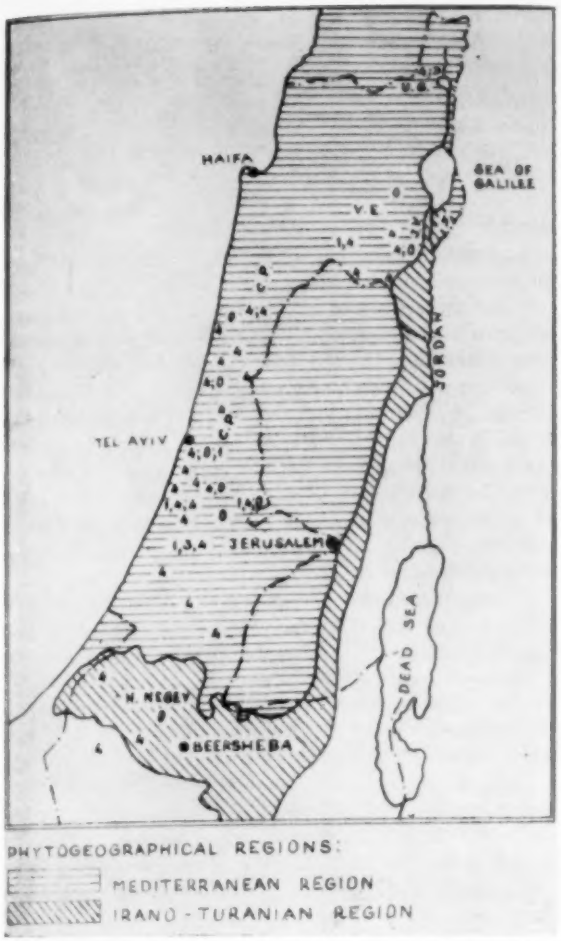

Fig. 1. Distribution of physiologic races of potato late blight fungus in Israel in 1954–57. Numbers designate the specified race; initials stand for regions in which the identified samples were collected. C.P., Coastal Plain; J.V., Jordan Valley; U.G., Upper Galilee; V.E., Valley of Esdraelon. Phytogeographical regions delineated according to Zohary (24), with slight modifications.

Table 1 with the pertinent data summarized by Black (3, Table 2) reveals that the races prevailing in Israel in spring of 1955 were identical to the races predominating in North Ireland in 1954, and probably also in Scotland.[3] Degree of agreement is somewhat less when the frequency of races occurrence is computed. Races 4 and 0 in spring of 1955 in Israel amounted to 73% and 27%, respectively, while in North Ireland in 1954 race 4 comprised 96% and race 0, 4% of the investigated isolates (3).

Pathogenicity of potato isolates on tomato differential varieties.—Observations from several countries emphasize the danger involved in disseminating inoculum from blighted potato fields to nearby tomato plantings (14). On the other hand, reference is made to instances where healthy tomatoes could be cultivated alongside potatoes affected by *P. infestans* (21). Field surveys conducted in Israel throughout 1951–57 conform with both of these apparently conflicting results.

Elucidation of interrelation between the late blight diseases on both crops is of great practical significance, particularly in countries like Israel, where potatoes and tomatoes are often grown in close proximity.

Berg (1) and Giddings and Berg (7) in the United States, and Röder (17) in Europe found potato and tomato races as distinct biological entities. This opinion, contested by Mills (15), has received new support in recent years (22). Furthermore, it has been demonstrated that both the potato and tomato races are complex units (2, 22), consisting of numerous components that vary in virulence on potato and tomato suscepts.

The following studies were undertaken to gain better understanding of the relation between potato and tomato late blight diseases under local conditions.

MATERIALS AND METHODS.—The procedure adopted was similar to that described above in identifying races on potato differential hosts. It was ascertained that tomato leaves removed from 9–11-week-old seedlings, since they do not rot easily, are even more amenable than potato foliage to detached-leaf culture.

To tomato differential varieties was added our common variety Marmande, of commercial importance in Israel. This variety was found to be equivalent to the recessive variety Rutgers as a differential host. The differential set comprised the following varieties and accessions: Rutgers and Marmande; W.Va. 106, W.Va. 36-1, W.Va. 700. Details related to genetic constitution of the listed accessions are discussed elsewhere (6, 22).

For uniformity, the nomenclature proposed by Gallegly and Marvel (6) was applied with minor modifications. Potato isolates nonpathogenic or slightly pathogenic on all tomato differentials including Rutgers and Marmande are labeled here as T_{00}. This race corresponds to Berg's original "potato race" (1). Isolates virulent solely on Rutgers and Marmande represent the common tomato race 0, and are denoted T_0, as suggested by Gallegly and Marvel (6). For the race virulent on Marmande, Rutgers, and accession W.Va. 106, but innocuous on W.Va. 36-1 and W.Va. 700, the designation T_1 was retained, while isolates pathogenic on Rutgers, Marmande, W.Va. 106, and W.Va. 36-1, but avirulent on W.Va. 700, were named T_1a. This designation is in agreement with the hypothesis postulating that both accessions, W.Va. 106 and W.Va 36-1, have in common a single dominant factor for resistance, TR_1, combined in W.Va. 36-1 with multiple genes (6) that confer additional protection. Therefore Gallegly and Marvel prefer to consider isolates signified by us as T_1 and T_1a as one race, and denote it T_1 (6). Some of the isolates studied in our tests also attacked W.Va. 700. Since the genetic nature of resistance displayed by W.Va. 700 is still unknown, the isolates are tentatively referred to as T?. Obviously, further progress in knowledge of the genetic

[3] Data available for Scotland relate the race situations in 1955 and 1955–56 (3). It is most likely, that races 4 and 0 were present there in 1954 also.

makeup of the differentials may involve alterations in the employed nomenclature.

RESULTS.—Twenty-five isolates of *P. infestans* collected from the commercial potato varieties in different regions of the country consisted of races or subraces in the following order of prevalence: T_{00}, 36%; T_0, 28%; T_1, 16%; T_1a, 16%; and T?, 4%.

The common occurrence of race T_{00} in Israel may explain the fact that healthy tomato crops are sometimes grown alongside blighted potatoes. Conversely, the high prevalence on potatoes of races capable of attacking our commercial tomato varieties makes it imperative to maintain proper distances between potato and tomato plantings.

Further tests were devised to determine the relations between potato race and tomato race characteristics of the potato isolates of the fungus. The results are summarized in Table 2. Analysis of the data presented in Table 2 shows that 19 potato isolates identified on potato differentials as race 4 may be subdivided, on the basis of tomato race characteristics displayed on tomato differential hosts, into the following races or subraces: T_{00}, T_0, T_1, and T_1a. They consequently corroborate the conclusion that there is "no correlation between potato-race characteristics and reaction on tomato hosts" (22). Hence, the suggestion to establish separate terminology for designation of potato and tomato races of *P. infestans* (6, 22) seems justified.

TABLE 2.—*Relations between tomato-race and potato-race characteristics of 23 potato isolates of P. infestans*

International potato race	No. of isolates with the following tomato-race characteristics:			
	T_{00}	T_0	T_1	T_1a
0				2
4	9	7	2	1
1, 4			2	

DISCUSSION AND CONCLUSIONS.—One of the main objectives of the presented study was to elucidate how the basic factors such as the physiologic specialization of the pathogen in the countries of origin of our seed tubers, and the specific climatic conditions of Israel affect the composition of races of *P. infestans* parasiting potatoes in our fields. Since the pathogen is tuber-transmitted, it was a good reason to anticipate correlation and affinity between physiologic races attacking the domestic potato crops and the potato cultivations in Scotland and N. Ireland, from which come 90% of seed material for our main crop grown in spring.

On the other hand, it was justified to assume that climatic conditions prevailing in the definitely arid Beersheba district of the N. Negev, or the ecological environment of the farm settlements in the Jordan Valley situated about 200 m below the sea level may alter the population trends of the physiologic races as compared with N. Europe and other parts of the globe. For similar reasons one could expect that the conspicuous variability of climatic conditions in Israel, as reflected in differences in natural vegetation (Fig. 1), would result in pronounced diversity of races in the regions concerned.

It was ascertained, however, that the physiologic races of *P. infestans* in Israel exhibit population shiftings similar to those recorded in Europe, N. America, and S. Rhodesia. Contrary to expectations (8), and for unknown reasons (3), the specialized race 4 became predominant here and in the above-mentioned parts of the world to replace the common race 0. Furthermore, race 4 appears to be widespread in all potato-growing regions of Israel, despite the striking differences in their climates.

Black stressed the importance of investigating the effect of climatic factors on the "vigor and reproductive powers" of physiologic races (2). If climatic areas and physiologic races could be correlated and the pattern of these relations determined, it may be possible to cultivate varieties according to their genetic constitution in regions where they would be least affected by the disease. The results of our studies (Fig. 1) seem to disprove the existence of such a correlation between distribution of physiologic races of the parasite and climatic conditions.

Perhaps the clue to understanding of the unrestricted expansion of race 4 in Israel should be sought in the complex nature of a physiologic race, which consists of distinct biological entities (2) acting alike on a limited standard assortment of differential genotypes, but diverse in their requirements for nutrition (9) or temperature (12).

Thus, it is conceivable that subraces or biotypes of race 4, prolific and abundant on potatoes in the Jordan Valley or in the N. Negev, were already present in minute quantities in N. Europe. Presumably, the selective influence of the new environment brought them from obscurity to prominence.

The factors responsible for greater persistence in nature of certain physiologic races, and simultaneous decline or elimination of others, are not sufficiently clear (19). The experience gained with *P. infestans* in the last several years confirms the opinion that there is no a priori reason to assume that the more specialized races are less aggressive than the common race 0 on the recessive potato varieties (13). Black demonstrated in 1952 that race 2,4 and possibly also race 4 may survive, together with race 0, on recessive potato varieties (2, Table 3).

Identification studies conducted with potato isolates on potato and tomato differential sets confirm the conclusions reached by Wilson and Gallegly (22) emphasizing the lack of relationship between genetic factors controlling late blight resistance in both crops. For example, 19 potato isolates determined as race 4 on differential potato varieties could be readily subdivided into the principal tomato blight races currently recognized.

The practical implications of the reported findings are obvious. Firstly, they explain the existence of healthy tomato fields alongside badly stricken potatoes. This may happen when the latter crop is invaded by race T_{00}, pathogenic solely on potatoes. Secondly, the established fact that the recessive tomato varieties, including our main variety Marmande, are vulnerable

to over 60% of the isolates identified on differential tomato varieties, makes it imperative to maintain proper distances between potato and tomato plantings.

In the breeding program for potato late blight resistance, particular attention should be given to the major genes: R_1, R_2, and R_3. The protection attained may be more enduring if these major genes will be combined with genetic factors for field resistance (3).

Chances for combating tomato late blight in Israel by breeding are not promising at present, since physiologic races capable of attacking the sources of resistance incorporated in the accessions, W.Va. 106, W.Va. 36-1, and W.Va. 700, are not uncommon in this country. It is noteworthy that these specialized races seem to be relatively more prevalent in Israel than in the United States, the Netherlands, and Scotland (22, Table 1). The origin of the virulent tomato races in Israel is unknown and deserves to be studied because of its great significance. Also, a search for a more desirable parental material, obviously, is indicated.

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LITERATURE CITED

BERG, A. 1926. Tomato late blight and its relation to late blight of potato. West Va. Univ. Agr. Expt. Sta. Tech. Bull. 205, 31 p.

2. BLACK, W. 1952. A genetical basis for the classification of strains of Phytophthora infestans. Proc. Roy. Soc. Edinburgh, Sect. B., **45**: 36-51.
3. BLACK, W. 1957. Incidence of physiological races of Phytophthora infestans in various countries. Scot. Soc. for Res. in Plant-Breeding. Ann. Rept. p. 43-49.
4. BLACK, W., C. MASTENBROEK, W. R. MILLS, AND L. C. PETERSON. 1953. A proposal for an international nomenclature of races of Phytophthora infestans and of genes controlling immunity in Solanum demissum derivatives. Euphytica **2**: 173-178.
5. BORDOUKOVA, M. V., AND O. D. BELOVA. 1958. Razrabotka sovietskimi utchonymi mier borby z bolezniami kartofeli (Russian: Work of Soviet scientists on control measures of potato diseases). Kartofel, Jan.-Feb.: 14-18.
6. GALLEGLY, M. E., AND M. E. MARVEL. 1955. Inheritance of resistance to tomato race 0 of Phytophthora infestans. Phytopathology **45**: 103-109.
7. GIDDINGS, N. J., AND A. BERG. 1919. A comparison of the late blights of tomato and potato. A preliminary report. Phytopathology **9**: 209-210.
8. GRAHAM, K. M. 1955. Distribution of physiological races of Phytophthora infestans (Mont.) De Bary in Canada. Am. Potato J. **32**: 277-282.
9. HODGSON, W. A. 1958. Growth of four races of Phytophthora infestans (Mont.) de Bary in synthetic media. Can. J. Plant Sci. **38**: 145-154.
10. HOWATT, J. L., AND P. N. GRAINGER. 1955. Some new findings concerning Phytophthora infestans (Mont.) De By. Am. Potato J. **32**: 180-188.
11. KAMMERMANN, N. 1950. Undersökningar röraude potatisbladmöglet, Phytophthora infestans (Mont.) De By. Part I. Statens Växtskyddsanstalt, Medd. 57. Stockholm (Swedish-German summary).
12. LARANCE, R. S., AND W. J. MARTIN. 1954. Comparison of isolates of Phytophthora infestans at different temperatures. (Abstr.) Phytopathology **44**: 495.
13. MASTENBROEK, C., AND TH. DE BRUIN. 1955. Het voorkomen van physio 4 van Phytophthora infestans in Nederland. (Dutch, with an English summary: The occurrence of race 4 of Phytophthora infestans in the Netherlands). Tijdschrift over Plantenziekten **61**: 88-92.
14. MILLER, P. R., AND M. J. O'BRIEN. 1955. Tomato late blight: its world distribution and present status. U.S. Dept. Agr. Plant Disease Reptr. Suppl. 231, 89 p.
15. MILLS, W. R. 1940. Phytophthora infestans on tomato. Phytopathology **30**: 830-839.
16. MULLER, K. O., J. C. CULLEN, AND M. KOSTROWICKA. 1955. Testing "true resistance" of the potato to blight Phytophthora infestans. J. Nat. Inst. Agr. Botany **7**: 341-354.
17. RÖDER, K. 1935. Untersuchungen über die Phytophthorakrankheit (Phytophthora infestans) der Tomate. Unter besonderer Berücksichtigung der biologischen Spezialisation des Erregers. Phytopathol. Z. **31**: 589-614. (German).
18. SCHICK, R., E. SCHICK, AND M. HAUSSDÖRFER. 1958. Ein Beitrag zur physiologischen Spezialisierung von Phytophthora infestans. Phytopathol. Z. **31**: 225-236. (German).
19. STAKMAN, E. C., AND J. G. HARRAR. 1957. Principles of Plant Pathology. The Ronald Press Co., New York. 581 p.
20. STEVENSON, F. J., AND R. V. AKELEY. 1953. Control of potato diseases by disease resistance. Phytopathology **43**: 245-253.
21. WALKER, J. C. 1952. Diseases of vegetable crops. McGraw-Hill Book Co. Inc., New York, Toronto, London. 529 p.
22. WILSON, J. B., AND M. E. GALLEGLY. 1955. The interrelationship of potato and tomato races of Phytophthora infestans. Phytopathology **45**: 473-476.
23. ZAAG, D. E. VAN DER. 1956. Overwintering en epidemiologie van Phytophthora infestans, tevens enige nieuwe bestrijdingsmogelijkheden. (Dutch, with an English summary: Overwintering and epidemiology of Phytophthora infestans, and some new possibilities of control). Tijdschrift over Plantenziekten **62**: 89-156.
24. ZOHARY, M. 1955. Geobotany. Syfryath Hapoaliym—Maanyth. 589 p. (Hebrew).

FUNGISTATIC ACTIVITY OF CAPTAN IN PEA SEEDLINGS AFTER TREATMENT OF THE SEEDS OR ROOTS OF SEEDLINGS [1]

V. R. Wallen [2] and I. Hoffman [3]

SUMMARY

Pea seedlings grown from seed treated with Orthocide 75 (75% captan) were slightly less susceptible to *Ascochyta pisi*, cause of leaf and pod spot of peas, than seedlings grown from untreated seed. A fungistatic substance was demonstrated in the treated seedlings by bioassay tests on an agar medium with *Saccharomyces pastorianus*, the test organism. By chemical analyses, a maximum of 0.25 ppm of captan was found in the aboveground parts of such seedlings. As this amount of captan is not considered sufficient to reduce infection by *A. pisi*, it is suggested that breakdown products not detected by chemical analysis caused the fungistatic action in the foliage. When captan was dissolved in acetone before being supplied to the pea seedlings, more of the chemical was detected in the foliage and greater chemotherapeutic protection was afforded to seedlings from *A. pisi* than when captan was added without a solvent. Captan was detected chemically in treated seeds for 9 weeks after the seed was sown in soil.

In previous tests (10) 4 distinct races of *Ascochyta pisi* Lib., cause of leaf and pod spot of peas, were distinguished on the basis of reaction of 7 differential varieties of peas. In an effort to increase seed germination, all seeds were treated before planting with Orthocide 75 (75% captan). Seedlings from captan-treated seed were found in repeated trials to be less susceptible to infection with the 4 races of *A. pisi* than were seedlings grown from untreated seed.

Captan (*N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide) has proved outstanding in several trials as a seed treatment to protect peas from seed rot and damping-off (1, 11, 12, 13). Napier et al (8) investigated the systemic activity of captan sprayed on broad beans. Sprayed on the dorsal surfaces of leaves, it protected the ventral surfaces from infection by *Botrytis fabae* Sardina, the cause of chocolate spot. They also found that captan could be absorbed through the roots and protect the foliage against the pathogen. May et al (7) found, in preliminary trials, that residues of acetone extracts from leaves or stems of elms growing in captan-treated soil were active against *Ceratocystis ulmi* (Buis.) C. Moreau in bioassay tests.

This paper presents the results of bioassay and chemical analysis to determine possible systemic activity in pea seedlings following application of captan, either as a seed treatment or in solution as a root drench.

METHODS AND RESULTS.—*Bioassay.*—A bioassay test was developed to measure the fungicidal activity of pea seedlings grown from seed treated with Orthocide 75. Seedlings grown in the greenhouse or in the field were assayed for fungistatic activity on an agar medium with *Saccharomyces pastorianus* Hansen as the test organism. Preparation of the agar used for the assays has been described by Whiffen (14). *S. pastorianus* was selected as the test organism because it proved to be more sensitive and gave clearer, better-defined zones of inhibition than any of a number of other organisms tested. Cells of *S. pastorianus* were obtained by growing the organism in shake culture in a liquid medium similar to the assay medium, but without agar. The shake cultures were diluted with phosphate buffer to a concentration of 200,000 cells per ml and stored in a refrigerator at 10°C.

Before assay, whole seedlings, or individual roots, stems, or leaves, were sterilized by exposing them for 24 hours to the fumes of propylene oxide in closed Petri dishes. This treated material was then transferred to the agar medium seeded with the test organism. After 24 hours' incubation at 30°C. the greatest width of the zone of clear agar across a portion of the stem tissue was measured to indicate the magnitude of fungistatic activity.

In a second procedure the treated seedlings were ground in tissue grinders containing 1 ml of chloroform. Filter paper disks (Schleicher and Schuell No 740E) were immersed in the chloroform extracts until saturated, and then air dried and transferred to the surface of agar freshly seeded with the test organism. After 24 hours' incubation, measurements of the activity of extracts of plant material were recorded as the size of the clear zone around the filter-paper disks.

Analytical procedures.—The method used was essentially that of Kittleson (3) as modified by Taylor and Klayder (9). The fresh material was weighed, placed in a large separatory funnel, and covered with benzene. Several hours were allowed for the captan to dissolve, during which time the funnel and contents were shaken occasionally. The benzene portion was recovered after filtration on a Whatman No. 12, 24-cm folded filter paper. Two benzene washings of the sample were similarly filtered and added to the main portion. The benzene solution was transferred to a separatory funnel, shaken vigorously with 10 g of cleanup mixture (2:1:1 nuchar–hyflo-super-cel–anhydrous-sodium-sulphate) and then recovered after filtration with suction. A second cleanup was usually

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[3] Analytical Chemistry Research and Services, Ottawa, Ontario.

necessary to ensure complete color removal. The benzene was transferred to a large beaker, several glass beads were added, and the volume was reduced to about 5 ml by evaporation with an infrared heater. The concentrated solution was recovered in a 25 × 200-mm test tube after filtration on a Whatman No. 42 7-cm paper. A small amount of benzene, used to wash the beaker, was added to the test tube through the filter paper. Resorcinol (0.5 g) was added, and fusion was carried out as described by Taylor and Klayder. In some cases it was necesary to filter the solution after cooling in water at room temperature before diluting to the 25-ml mark with acetic acid.

Transmittance measurements were made on a Warren Spectrachord, using 1-cm cells. These were compared with standard curves. Some of the extracts displayed continuously changing transmittance values with changing wave length, without an absorption maximum. In such cases, if single absorbance values were taken at 425 mμ, an erroneous interpretation indicating the presence of captan could be made, as, for example, curve B in Fig. 1. This difficulty does not arise with the present instrument, since absorption maxima centered at 425 mμ can be seen at a glance. In the case of curve C the maximum was not very pronounced. Conclusive proof of the presence of captan was obtained by reading against a blank obtained from untreated peas instead of against pure glacial acetic acid (curves A and D).

Systematic fungistatic activity by bioassay.—Seeds of the pea variety Chancellor were treated with Orthocide 75, both as a dust at the recommended rate of 2½ oz. per 100 lb., and as a slurry at 1¾ oz. per 100 lb. Forty-eight hours after treatment the peas were sown in soil in the greenhouse or placed between moist paper towels. At emergence, and at 1 and 2 weeks after emergence, the seedlings were dug up, cut in 2 sections at the soil line, washed in running tap water, dried, and assayed immediately or frozen for future assay. Seedlings were assayed by both the paper-disk and direct-plating methods. Five seedlings each of control, dust-treated, and slurry-treated were assayed by each method. All Petri dishes were incubated 24 hours at 30°C and examined for visible zones of inhibition.

It was noted that small zones of inhibition occurred around the untreated seedlings, but larger zones were apparent around the captan-treated seedlings and around filter-paper disks that had been impregnated with the filtrate from captan-treated seedlings. When the direct-assay method was used, the size of the zones varied from 0 to 35 mm for untreated seedlings and from 28 to 43 mm for captan-treated seedlings. The important difference in captan assays was the absence of any growth of the test organism immediately adjacent to the young seedlings. In control seedling assays a trace of growth of *S. pastorianus* usually occurred near the attachment of the seed to the hypocotyl within the zone of inhibition. With the disk assay procedure, no clear zones of inhibition occurred around the extracts from control seedlings, but zones up to 28 mm in diameter were noted with extracts from captan-treated seedlings (Fig. 2). A natural inhibitor must occur within normal pea seedlings, because control-seedling assays showed some zones of inhibition. In further tests this inhibition in control-seedling assays partially masked the effect of the fungistatic substance in captan-treated seedlings. It was therefore decided that chemical assays would be necessary to characterize the fungistatic substance present in captan-treated seedlings.

Systemic fungicidal activity by chemical analysis.—Seed of the pea variety Improved Thomas Laxton was treated with Orthocide 75 as a dust at the recommended rate of 2½ oz. per 100 lb. of seed. Forty-eight hours after treatment, the seeds were sown in soil in a greenhouse bench. At emergence, and at weekly intervals for 9 weeks, young seedlings were dug and excised approximately 1 in. above the soil line. The remaining portion was washed in fast-running tap water so that all the soil and as much as possible of the surface captan was removed. After being washed, both the

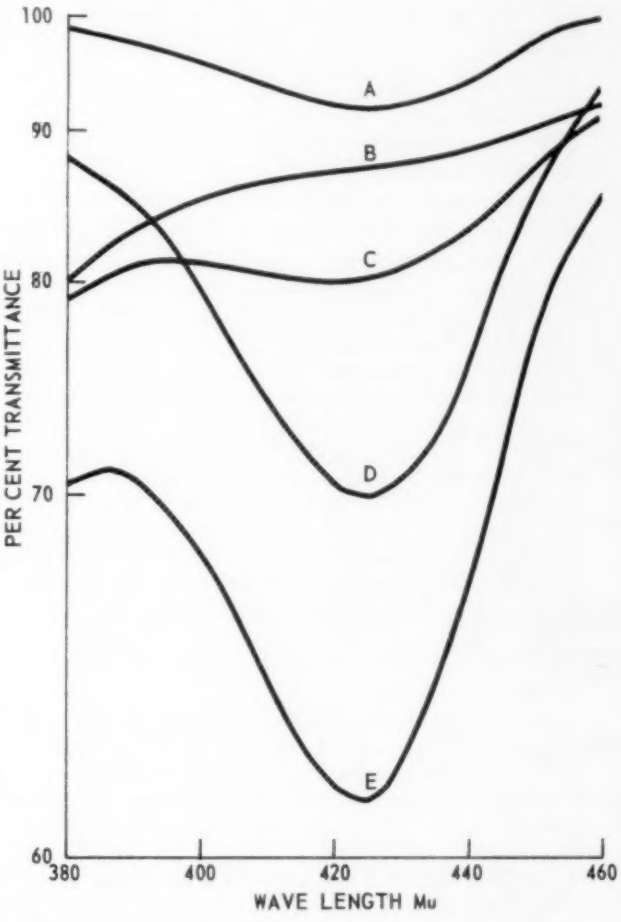

Fig. 1. Transmittance measurements of various captan extracts. **A)** Peas grown in Hoagland's solution (H's) + captan vs. untreated peas grown in (H's). **B)** Peas grown in (H's) vs. glacial acetic acid (G.A.). **C)** Captan-treated peas + (H's) vs. G.A. **D)** Peas grown in (H's) + captan dissolved in chloroform vs. peas grown in (H's). **E)** As in D, but vs. G.A.

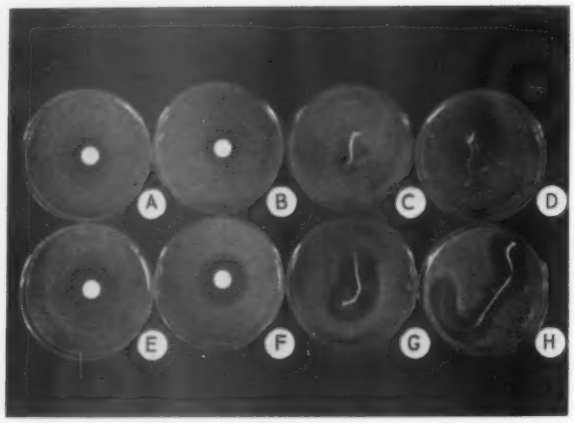

Fig. 2. Inhibition zones produced by pea seedlings, and extracts of seedlings seed treated with Orthocide 75 (75% captan) and grown in soil in the greenhouse. **A-D)** untreated, **A)** plumule and hypocotyl, paper-disk assay, **B)** root, paper-disk assay, **C)** hypocotyl and plumule, direct assay, **D)** root, direct assay, **E-H)** captan treated, **E)** hypocotyl and stem, paper-disk assay, **F)** root, paper-disk assay, **G)** hypocotyl and plumule, direct assay, **H)** root, direct assay.

lower and upper portions of the seedlings were weighed and separately analyzed chemically for captan content by the described method.

In two separate analyses a trace and 0.25 ppm of captan were found at emergence in the aboveground portion of captan-treated seedlings grown in the greenhouse. Analysis of the belowground portions of the young plants, consisting of the cotyledons, seed coat, root, and that part of the shoot belowground, revealed that captan was present throughout the duration of the 9-week test period. The average weight of captan per seed was 280 μg before planting. At emergence this amount was reduced to 7 μg; one week after emergence 2 μg of captan was present, and the second week after emergence, 0.3 μg. Nine weeks after emergence less than 0.1 μg of captan was recovered. At 4 weeks the cotyledons started to deteriorate, and, although they were washed very carefully, traces of moss used in the compost were present. This material in the vicinity of the seeds may have contained some of the captan found in the 4–9-week period.

In addition to the greenhouse trials, a field trial was conducted. Seed of Improved Thomas Laxton peas was treated at the recommended rate and sown in rod-row plots in the field. At weekly intervals after emergence, the young plants were excised 1 in. above the soil line, and the upper portion was bulked into one sample, and the lower portion bulked into a second sample. In preliminary trials with the aboveground portions of one- and two-week-old seedlings, captan could not be detected by the described chemical method. Captan was found in the belowground portions of the plants. More captan could be detected if the plant material was assayed without preliminary drying. When sand was used to grind this material and benzene used to extract captan, a colloidal formation developed that made testing for captan impossible.

Root absorption of captan.—An experiment was carried out to determine if captan applied in suspension or in solution could be absorbed by the root system and translocated to the aboveground parts of young pea seedlings. Four lots of 48 crocks containing quartz sand were sown with untreated pea seed, variety Improved Thomas Laxton. The crocks were watered daily until the young seedlings emerged. The seedlings were then supplied at two-day intervals with Hoagland's solution (2), 200 ml to each crock, until the seedlings were about 2 in. high. At this time, 4 treatments were applied to the quartz sand by means of a glass funnel thrust 1 in. into the sand. The glass funnel was used primarily to avoid any contamination by captan to the aboveground parts of the plants. The treatments were as follows: Hoagland's solution, Hoagland's solution + captan, Hoagland's solution + captan dissolved in acetone, and Hoagland's solution + captan dissolved in chloroform. The final concentration of acetone and chloroform in Hoagland's solution was 2%. The final concentration of captan was 100 ppm. Three applications of 200 ml of the above solutions were supplied to the young seedlings at 48-hour intervals. Forty-eight hours after the final application, the seedlings were excised 1 in. above the soil line, separated into above- and belowground portions, washed, bulked, weighed, and assayed chemically for captan content.

Chemical analysis revealed that captan was present in the aboveground parts of the plants in the three series in which captan was incorporated with Hoagland's solution. When captan was dissolved in acetone and chloroform, 0.85 ppm captan and 0.44 ppm captan were found, respectively. Only 0.04 ppm captan was found when captan was added to Hoagland's solution without any solvent (Fig. 1).

Infection studies.—In previous tests, it had been shown as a result of certain treatments that captan was translocated from the roots of young pea plants into the aboveground parts of the plant. It was now decided to find out if the application of captan to the roots of pea seedlings offered any protection against the development of leaf and pod spot caused by *A. pisi*.

Improved Thomas Laxton pea seedlings were grown in quartz sand in ½-gal. earthenware crocks at the rate of 10 seedlings per crock. The crocks were watered until the young seedlings emerged, then supplied with Hoagland's solution until the seedlings were about 2 in. high. The plants were then sprayed with a spore suspension of *A. pisi* in sterile tap water and placed in a growth chamber. The growth chamber provided adequate light for growth and was maintained at a temperature of 18°C and a relative humidity of 85% for the duration of the test. At inoculation, and 48 hours and 96 hours afterward, 4 treatments similar to those used in the previous test on root absorption were applied. Two weeks after inoculation the seedlings were examined for lesions by *A. pisi*. The treat-

TABLE 1.—*The effect of various treatments of captan applied to the roots of pea seedlings on the development of Ascochyta pisi*

Treatment	Number of seedlings [a]	Number of seedlings with		Number disease-free
		Fruiting lesions	Non-fruiting lesions	
Hoagland's solution	96	42	24	30
Hoagland's solution + captan	94	16	24	54
Hoagland's solution + captan + chloroform	80	40	24	16
Hoagland's solution + captan + acetone	98	0	10	88

[a] Total of 4 replications.

ments used and the results of this test are shown in Table 1.

Forty-two of 96 seedlings supplied with Hoagland's solution only developed fruiting lesions of *A. pisi*. Sixteen of 94 seedlings supplied Hoagland's solution + captan developed fruiting lesions. Chloroform was phytotoxic to the young plants, many of which wilted and in some cases died. Disease development was saprophytic on this material. Non-fruiting lesions developed on 24 seedlings each for the Hoagland's, Hoagland's + captan, and the Hoagland's + chloroform + captan treatments. The best chemotherapeutic protection was afforded when captan was dissolved in acetone incorporated into Hoagland's solution and supplied to the young plants.

DISCUSSION.—The data show that a fungistatic substance that checks the growth of the fungus *A. pisi* is present in pea seedlings grown from seeds treated with captan. By bioassay of the aboveground parts of the plant, the presence of a fungistatic substance that inhibited the growth of *S. pastorianus in vitro* was demonstrated. As a natural inhibitor was present in normal pea seedlings and slightly active against the test organism, chemical analysis was necessary to find if captan was the fungistatic substance. By chemical analysis of treated material a maximum of 0.25 ppm of captan was found. As only captan was identified in total, breakdown products may be responsible for this fungistatic activity. Kittleson (4, 5) stated that the *N*-trichloromethylthio group is the active portion of the captan molecule. Lukens and Sisler (6) reported that they considered a combination of thiophosgene with free sulfhydryl, amine, hydroxyl, or carboxyl groups of the host cell, or a combination of the trichloromethylthio group through the free bond of sulfur to vital groups in the cell as the chemical reactions responsible for the fungtoxicity of captan. As captan is insoluble in water it would not be expected to pass the cell barrier to any extent; however, a fungistatic substance was present in captan-treated seedlings.

The fact that captan dissolved in acetone or chloroform was more readily absorbed by plant roots than was captan in a water suspension may be of practical significance in applying captan to foliage for protection against different pathogens. If smaller amounts of captan can be applied in solution the danger from toxic residues could be reduced. Further work is warranted on this phase of the problem.

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LITERATURE CITED

1. DE ZEEUW, D. J., A. L. ANDERSEN, AND G. E. GUYER. 1956. Comparison of fungicide and fungicide-insecticide seed treatments of peas and beans. Phytopathology **46**: 10.
2. HOAGLAND, D. R., AND D. I. ARNON. 1938. The water-culture method for growing plants without soil. Univ. Calif. Agr. Expt. Sta. Circ. 347, p. 36-39.
3. KITTLESON, A. R. 1952. Colorimetric determination of N-Trichloromethylthiotetrahydrophthalimide. Anal. Chem. **24**: 1173-1175.
4. KITTLESON, A. R. 1952. A new class of organic fungicides. Science **115**: 84-86.
5. KITTLESON, A. R. 1953. Preparation and some properties of N-(trichloromethylthio) tetrahydrophthalimide. J. Agr. Food. Chem. **1**: 677-679.
6. LUKENS, R. J., AND H. D. SISLER. 1958. Chemical reactions involved in the fungitoxicity of captan. Phytopathology **48**: 235-244.
7. MAY, C., J. G. PALMER, AND E. HACSKAYLO. 1958. Inhibition of growth of Ceratocystis ulmi in vitro by residues from extracts of soils and of plants growing in soils treated with captan or Orthocide 50W. Plant Disease Reptr. **42**: 696-702.
8. NAPIER, E. J., A. RHODES, D. I. TURNER, J. TOOTILL, AND A. DUNN. 1957. Systemic action of captan against Botrytis fabae (chocolate spot of broad bean). J. Sci. Food Agr. **8**: 467-474.
9. TAYLOR, D. M., AND T. J. KLAYDER. 1957. Report on captan on surface of fruits and vegetables. J. Assoc. Offic. Agr. Chemists **40**: 219-224.
10. WALLEN, V. R. 1957. The identification and distribution of physiologic races of Ascochyta pisi Lib. in Canada. Can. J. Plant Sci. **37**: 337-341.
11. WALLEN, V. R., AND W. BELL. 1956. Treatment of vegetable seed for improved emergence—1955. Plant Disease Reptr. **40**: 129-132.
12. WALLEN, V. R., J. K. RICHARDSON, L. CINQ-MARS, AND W. BELL. 1957. Treatment of vegetable seed for improved emergence—1956. Plant Disease Reptr. **41**: 468-473.
13. WALLEN, V. R., M. A. WALLACE, AND W. BELL. 1955. Response of aged vegetable seed to seed treatment. Plant Disease Reptr. **39**: 115-117.
14. WHIFFEN, ALMA J. 1948. The production, assay, and antibiotic activity of actidione, an antibiotic from Streptomyces griseus. J. Bacteriol. **56**: 283-291.

PATHOGENICITY OF FUNGI ISOLATED FROM DISEASED COTTON SEEDLINGS [1]

N. D. Fulton and Katharina Bollenbacher [2]

SUMMARY

Twenty-two fungus isolates were tested individually for pathogenicity on the seed and seedlings of cotton. Included were an *Alternaria* sp., *Aspergillus flavus, A. niger,* a *Cladosporium* sp., *Colletotrichum gossypii,* a *Curvularia* sp., *Fusarium oxysporum, F. moniliforme, F. oxyporum* f. *vasinfectum,* a *Helminthosporium* sp., a *Penicillium* sp., *Pythium ultimum, Rhizoctonia solani,* a *Rhizopus* sp., *Sclerotium bataticola, S. rolfsii, Thielaviopsis basicola,* a *Trichoderma* sp., and *Verticillium alboatrum.* Pre-emergence killing was much more prevalent with *Rhizoctonia solani* and *Pythium ultimum* than with other fungi. Most isolates were more pathogenic in tests held at a constant temperature of 21°C than in tests held one week at 27°C and then changed to 16°C or at a constant temperature of 27°C. Only *Rhizoctonia solani, Pythium ultimum, Colletotrichum gossypii, Thielaviopsis basicola,* and *Aspergillus flavus* were highly pathogenic to cotton seedlings. Striking differences in pathogenicity were found among the various isolates of a particular species, except for *Aspergillus flavus. Fusarium* spp. were in general only moderately to mildly pathogenic. Other fungi were either nonpathogenic or only mildly so.

Securing an adequate stand of seedlings is often a major difficulty in cotton production. Growers usually plant an excess of seed to compensate for possible stand reduction from seedling disease. Such stand losses may make replanting necessary and may even result in yield losses because replanted fields are late.

The seedling disease complex of cotton has been a perplexing problem for many years. Atkinson (4) first adequately described damping-off of cotton in 1892, although the term "soreshin" had been used for the disease as early as 1855 (9). Numerous workers have shown that seedling disorders are caused by a complex of organisms (16, 20, 23, 24). Camp (6) reported *Rhizoctonia solani* Kuehn as a common cause of seedling disease, and Arndt (1, 2) demonstrated that *Colletotrichum gossypii* Southworth, *R. solani, Pythium ultimum* Trow, and several other fungi were pathogenic to cotton seedlings. Lehman (12) found that *C. gossypii* and *Fusarium* spp. were the most important pathogens of cotton seedlings in North Carolina. Rosen (18) reported that *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans. is pathogenic to cotton seedlings.

Surveys conducted by Miller and Weindling (14, 15) in 1939–40 showed that *C. gossypii* and *Fusarium* spp. were the most prevalent pathogens on cotton seedlings in the eastern and central Cotton Belt, including Arkansas. The present studies show that *R. solani* has become the most important cotton seedling pathogen in Arkansas and that *C. gossypii* now is relatively unimportant in the state.

Cotton seedling losses in the field are almost invariably associated with periods of cool, wet weather during the several weeks immediately following planting. Temperature studies carried out by various workers (3, 5, 8, 17, 19, 22) with known pathogens have indicated that temperatures lower than optimum for the host favor the pathogen. So far as the writers are aware, no temperature studies have been made with all the individual fungi associated with diseased cotton seedlings, nor have attempts been made to ascertain what part the so-called nonpathogenic organisms isolated from diseased seedlings play in the disease syndrome. The present studies were begun with the purpose of investigating the effect of environment on disease incidence, with both pathogenic and nonpathogenic fungi used singly and in selected combinations.

This initial investigation had 3 objectives: 1) to ascertain the relative prevalence patterns in the state for all fungi associated with cotton seedling disorders, with particular emphasis on the more destructive ones; 2) to determine the pathogenicity of all fungi recovered from diseased seedlings; and 3) to study the effects of temperature on individual fungal isolates and on their ability to incite disease.

MATERIALS AND METHODS.—The fungus isolates used in this study were obtained in 1954, 1955, and 1956 from diseased cotton seedlings collected from various areas in Arkansas—with 3 exceptions: one of the *Aspergillus flavus* Link isolates was obtained from the U. S. D. A.,[3] the *F. oxysporum* f. *vasinfectum* culture was isolated from a diseased cotton plant, and the *Sclerotium rolfsii* Sacc.[4] was isolated from tomato.

Isolations from cotton seedlings were made by removing sections from the margins of diseased and healthy hypocotyl tissue and submerging the sections in a 0.5% sodium hypochlorite solution for 2–5 minutes, depending on the size of the section. Plating was done in Petri dishes on potato-dextrose and on water

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[3] Isolated from fluorescent cotton fiber and supplied through the kindness of P. B. Marsh, Crops Research Division, ARS, U. S. D. A.

[4] Supplied through the kindness of J. P. Fulton, Arkansas Agricultural Experiment Station.

agar. Sections were incubated about 5 days at room temperature and were examined periodically. As soon as growth was sufficient, hyphal tips of individual fungi were transferred to agar slants for later identification. After identification, single-spore cultures were secured for all isolates except *R. solani*, *P. ultimum*,[5] *Sclerotium bataticola* Taub., and *S. rolfsii*. For the latter 4 fungi, hyphal-tip cultures were used. Temperature and pathogenicity studies were made on representative isolates.

Table 1 lists the fungi used in the tests. The Snyder and Hansen system (21) of nomenclature was used for the fusaria.

Temperature studies for all the isolates selected were carried out as a prerequisite to the pathogenicity experiments. Nine different temperatures were used in determining the maximum, optimum, and minimum growing temperatures of the microorganisms. A 7-mm disc of PDA inoculum was placed in the center of a Petri plate containing a standard volume of PDA. Each temperature series contained 4 replicates for each fungus, and every fungus was tested twice. The time of incubation varied from 24 to 112 hours, depending on the relative growth rates of the organisms involved. Growth rates were determined by measuring colony radius at the end of the incubation period.

Several methods of ascertaining the relative pathogenicity of the fungi were tried. The most satisfactory for greenhouse use was found to consist of growing the cotton seedlings in sand that had been sterilized and then inoculated with pure cultures of the respective fungi. One-gallon crocks and metal flats were tried as containers. The crocks proved much easier to handle and were used in most of the experiments. The same seed lot of Coker 100 W cotton variety was used for all experiments. The seed was machine-delinted and treated with Ceresan M. Inoculum was produced in a liquid medium consisting of a modified Richard's solution in which dextrose was substituted for sucrose and V-8 juice was added. In part of the pathogenicity tests the inoculum consisted of the fungus and the medium on which it grew; in the remainder of the tests the inoculum was made up of the fungus without the medium, which was replaced with an equal amount of sterile distilled water. In all cases the inoculum was thoroughly mixed with the sand just prior to planting and the seed were placed at a uniform depth of one-half inch. A standard volume of distilled water was added daily to each crock. After emergence the plants were given a balanced nutrient solution each day.

Three variations in temperature were used in the pathogenicity experiments: a dual temperature, 27–16°C, and 2 constant temperatures, 21° and 27°C. In the 27–16°C series the plants were grown 12 days at 27°C and then transferred to 16°C until termination of the test. The constant-temperature series were conducted at 21° and 27°C. respectively, until completion. Emergence counts were taken in all series at the end of 12 days. Per cent emergence was calculated by using the uninoculated controls as 100. At the end of 21 days the plants in each test were examined and rated for disease. Disease rating was by means of an index calculated by multiplying the number of plants with slightly discolored roots by 20, moderately injured plants by 40, severely injured plants by 60, and dead plants by 100, and dividing the sum by the total number of plants emerged in the uninoculated checks. Thus any fungus killing all seedlings would have a disease index of 100.

Uninoculated control crocks were set up with each series. In those tests where the fungus was separated from the medium on which it had grown, distilled water was added to the controls before planting; in tests where fungus and medium were both used as inoculum, plain sterile liquid medium was added to

TABLE 1.—*Pathogenicity at 3 temperatures (C) of 22 fungus isolates recovered from diseased cotton seedlings*

Fungus and isolate no.	Per cent emergence			Disease index		
	27-16°[a]	21°[b]	27°[b]	27-16°[a]	21°[b]	27°[b]
Uninoculated control	100	100	100	0	0	0
Alternaria sp.						
303 O	98	96	98	9	7	0
Aspergillus flavus						
411 O	93	90	88	19	38	61
A. niger						
421 O	93	98	97	13	4	6
Cladosporium sp.						
501 N	90	94	97	15	12	10
Colletotrichum gossypii						
611 O	94	89	96	96	58	77
612 Mn	90	85	94	69	82	38
Curvularia sp.						
701 F	99	98	94	5	0	7
Fusarium oxysporum						
2018 F	97	96	99	12	40	6
F. moniliforme						
212 Mn	100	99	92	8	24	21
F. oxysporum f. vasinfectum						
221 Mn	98	91	96	8	17	5
Helminthosporium sp.						
801 M	96	97	98	17	4	2
Penicillium sp.						
901 O	98	97	95	8	1	4
Pythium ultimum						
1501 O	14	0	9	100	100	92
Rhizoctonia solani						
111 A	2	20	38	100	100	99
118 O	19	1	14	99	100	97
1118 Pa	81	85	69	30	31	51
Rhizopus sp.						
1001 O	100	100	98	4	0	3
Sclerotium bataticola						
1112 H	99	99	100	5	2	1
S. rolfsii						
1121 F	91	83	100	13	24	1
Thielaviopsis basicola						
1313 O	97	100	99	53	80	50
Trichoderma sp.						
1201 O	100	93	96	3	7	6
Verticillium albo-atrum						
1411 Pk	96	100	98	11	1	3

[a] Fungus mycelium not separated from medium before inoculating sterile sand.

[b] Fungus mycelium separated from medium before inoculating sterile sand.

[5] Identification confirmed through the kindness of J. T. Middleton, University of California.

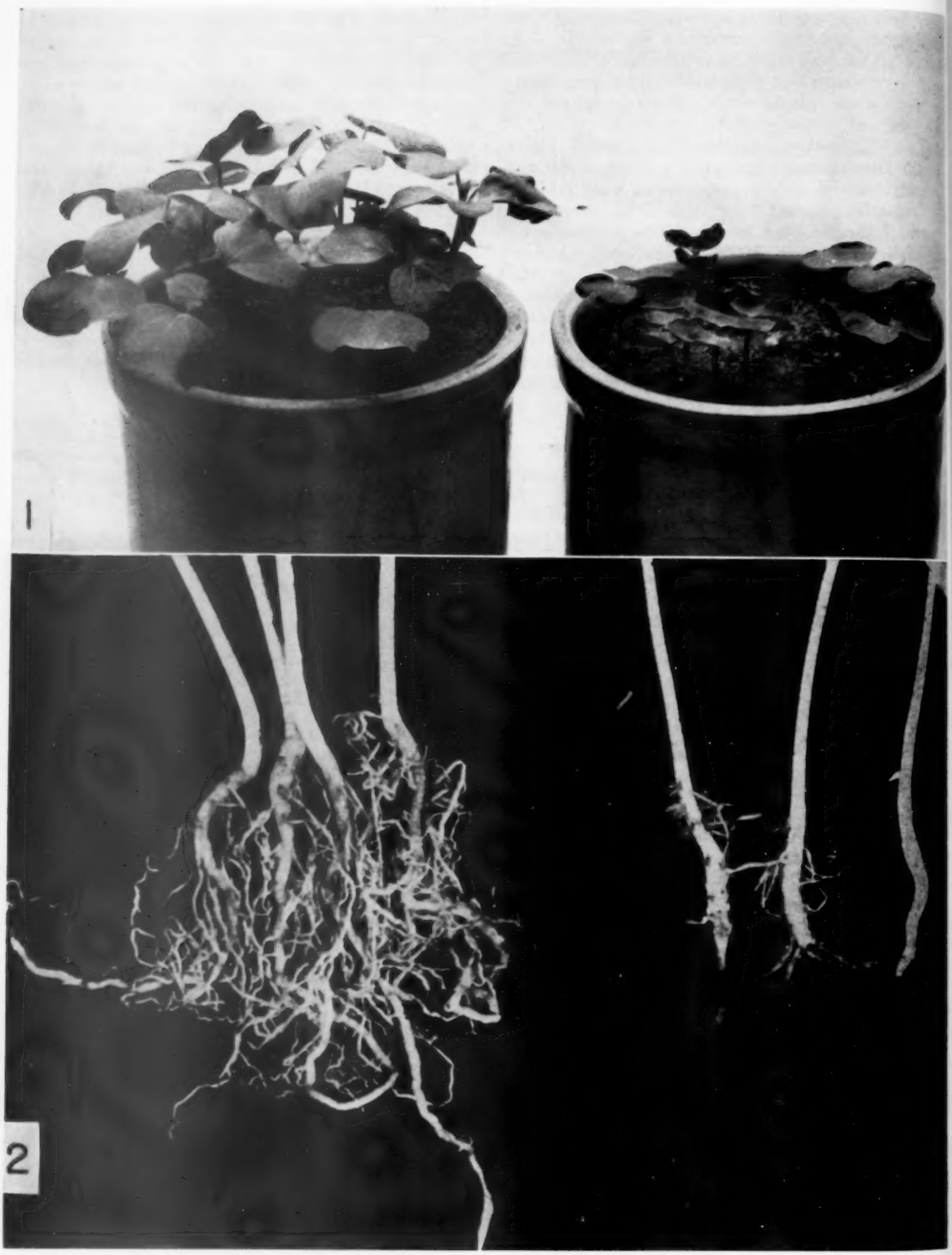

Fig. 1, 2. 1) Cotton seedlings grown in the greenhouse—**left,** in sterile sand; **right,** in sand infested with *A. flavus.* 2) Effect of *A. flavus* on the roots of cotton seedlings—**left,** plants grown in sterile sand; **right,** plants grown in sand infested with *A. flavus.*

TABLE 2.—*Frequency of occurrence in Arkansas of fungi pathogenic to cotton seedlings in 1955–58* [a]

Fungi	Per cent occurrence			
	1955	1956	1957	1958
Rhizoctonia solani	48	48	34	39
Pythium ultimum	2	1	2	7
Colletotrichum gossypii	1	6	1	3
Thielaviopsis basicola	2	0	0.5	5
Aspergillus flavus	1	1	0.5	1
Fusarium spp.	46	44	62	45

[a] Figures based on the more pathogenic fungi only. Several of the relatively nonpathogenic fungi, such as *Alternaria* spp. and *Penicillium* spp., were as prevalent as the fungi listed above.

the controls.

Each series of the pathogenicity tests contained 3 replicates and was repeated at least once to reduce errors in disease rating.

At the completion of each series, routine isolations were made to determine if the original inoculum could be recovered from its respective diseased host.

EXPERIMENTAL RESULTS.—*Frequency of occurrence of cotton seedling pathogens.*—The percentages of pathogenic fungi recovered during 1955–58 in Arkansas are shown in Table 2. During 1955 and 1956 *R. solani* was the most frequently isolated pathogen. During 1957 and 1958 the trend shifted somewhat and *Fusarium* spp. were more frequently recovered from diseased seedlings. *P. ultimum*, *C. gossypii*, *Thielaviopsis basicola* (Berk. & Br.) Ferr., and *A. flavus* were seldom isolated from diseased cotton seedlings. *Ascochyta gossypii* Sydow was recovered occasionally from seedlings, but was not included in the pathogenicity studies, because it was isolated more frequently from mature plants.

Relative pathogenicity of fungus isolates.—Twenty-two fungus isolates were tested individually for pathogenicity on the seed and seedlings of cotton. The results, summarized in Table 1, indicate that certain of the fungi are highly pathogenic and also that different isolates of the same species may vary widely in their pathogenicity to cotton seedlings. The most virulent fungi tested were *R. solani*, *P. ultimum*, *C. gossypii*, and *T. basicola*. These fungi killed a high percentage of seedlings, either by completely girdling the hypocotyl at the soil line or by preemergence damping-off. No consistent differences in lesion color or symptoms among the various pathogens were evident under the conditions of these experiments except that lesions produced by *T. basicola* were usually darker than those produced by other pathogenic fungi.

S. rolfsii and *Fusarium* spp. were moderately pathogenic, causing stunting, reduced root systems, and hypocotyl lesions that usually did not girdle the plants.

These studies established for the first time that *A. flavus* is moderately to highly damaging to cotton seedlings (Fig. 1). Symptoms were extreme stunting, almost complete loss of secondary roots, and the prevention of tap-root elongation (Fig. 2). Few if any lesions were found such as are produced by other pathogenic fungi. Three isolates were tested, and all

TABLE 3.—*Pathogenicity at 2 temperatures of 3 Aspergillus flavus isolates*

Fungus and isolate number	Per cent emergence [a]		Disease index [b]	
	21°C	27°C	21°C	27°C
Uninoculated check	100	100	0	0
Aspergillus flavus				
411 O[c]	90	88	38	61
412 Mn[d]	98	89	42	54
153[e]	89	98	52	67

[a] Emergence counts taken after 12 days.
[b] Disease indexes taken at the end of 21 days.
[c] Isolate taken from a diseased cotton seedling and found to produce fluorescence in cotton fiber.
[d] Isolate taken from a diseased cotton seedling.
[e] Isolate recovered from fluorescent cotton fiber by P. B. Marsh et al.

were about equally virulent. Table 3 summarizes these results. *A. flavus* was invariably recovered when sections of the diseased plants were surface-sterilized and incubated on agar.

Verticillium albo-atrum Reinke & Berth., *S. batatícola*, *Aspergillus niger* Van Tieghem, an *Alternaria* sp., a *Cladosporium* sp., a *Curvularia* sp., a *Helminthosporium* sp., a *Rhizopus* sp., a *Penicillium* sp., and a *Trichoderma* sp. were either nonpathogenic or only slightly pathogenic under the conditions of these experiments.

Influence of temperature on disease incidence.—A comparison of the growth rates of the pathogenic fungi and their ability to produce disease as affected by temperature is given in Table 4. The more virulent fungi appear to be able to produce disease over a wider temperature range than the moderately pathogenic fungi. At 21°C, cotton seedlings can be dam-

TABLE 4.—*Effects of temperature on growth in culture of various fungi and on their pathogenicity to cotton seedlings*

Fungus and isolate number	Temperature (°C)			
	Fungal growth		Disease severity	
	Minimum	Optimum	Minimum	Optimum
Rhizoctonia solani				
111 A	16	28	27	(27-16)
118 O	12	24-28	(27-16)	21
1118 Pa	12	24-28	(27-16)	27
Pythium ultimum				
1501 O	12	28	27	21
Colletotrichum gossypii				
611 O	12	28	21	(27-16)
612 Mn	16	28	27	21
Thielaviopsis basicola				
1313 O	12	24-28	27	21
Aspergillus flavus				
411 O	16	32	(27-16)	27
Fusarium oxysporum				
2018 F	16	28	27	21
F. oxysporum f. vasinfectum				
221 Mn	12	28	27	21
F. moniliforme				
212 Mn	12	28	(27-16)	21
Sclerotium rolfsii				
1121 F	16	32	27	21

[a] Dual temperature series.

aged by a greater variety of microorganisms in the soil complex. The results also indicate that the majority of the pathogens produce more severe disease symptoms at the lower temperatures.

Influence of rate of growth of pathogens on emergence of cotton seedlings.—Emergence percentages are summarized in Table 1. Almost all fungi tested apparently reduced emergence to some extent. In cases where fungus isolates were only slightly pathogenic, this emergence reduction was often the only evidence of disease. However, some of the most virulent fungi, e.g., *C. gossypii* and *T. basicola*, reduced emergence very little yet eventually killed most of the emerged seedlings. On the other hand, *R. solani* and *P. ultimum* killed most of the seedlings before emergence. Table 5 shows a direct correlation between rate of mycelial growth of the pathogens and reduction in per cent of seedling emergence, with the fastest-growing fungi showing the greatest virulence in the preemergence seedling stage.

Discussion.—The data in Table 1 present clear-cut evidence of a year-to-year change in the prevalence pattern of fungi pathogenic to cotton seedlings in Arkansas. Miller and Weindling (14, 15) reported *C. gossypii* as the most plentiful cotton-seedling parasite in Arkansas in 1939–40. The present study shows that *R. solani* has replaced *C. gossypii* as the most important offender in the state, and that the *Fusarium* spp. have maintained or increased their importance. *P. ultimum* and *T. basicola* appear to be relatively unimportant, since they were found only occasionally. *S. rolfsii* showed moderate pathogenicity to cotton seedlings, but it was rarely recovered from diseased seedlings and is not considered important. Although it is difficult to evaluate all the factors responsible for the above-cited change, the present general use of fungicides for seed treatment has undoubtedly helped eliminate *C. gossypii* as a disease factor in planting seed. As a soil inhabitant, *R. solani* is influenced much less by seed treatment than is *C. gossypii*, which is a seed-borne pathogen.

Many of the fungi isolated from diseased seedlings showed rather low pathogenicity to cotton seedlings. Some of those that produced no disease in the tests might be pathogenic under other environmental conditions or with seed of reduced viability. The role of these apparently nonpathogenic fungi in the seedling disease complex is still incompletely understood. Additional studies with fungal combinations and with cotton seed having varied degrees of vigor are in progress. It is hoped that these studies will help determine the influence on disease of those microorganisms usually found in diseased seedling tissue but which have little apparent direct effect on seed and seedlings of high vigor.

These tests have established *A. flavus* as a new pathogen of cotton seedlings. Marsh et al (13) reported that *A. flavus* produced fluorescence in cotton fiber and caused loss of fiber strength. One of the isolates carried in the present studies was a subculture of the isolate that Marsh found to produce a consistently high degree of fluorescence in artificial inoculation tests on cotton fiber. One isolate from cotton seedlings was employed in artificial inoculation tests in this study and produced the same type of fluorescence in cotton fiber as that described by Marsh. The virulence of all *A. flavus* isolates did not vary greatly in the seedling disease tests. Koehler and Woodworth (10) established that *A. flavus* can cause a virescence in corn seedlings. The present authors were unable to find any reference to pathogenicity of *A. flavus* to cotton seedlings. Whether *A. flavus* is very important in Arkansas as a seedling pathogen is questionable. It is, however, much more prevalent in the southwestern states, where it has been found associated with damaged cotton fiber.

Table 5.—*Rate of growth of pathogens related to emergence of cotton seedlings*

Fungus and isolate number	Pathogen[a] growth at 20°C (mm)	Seedling[b] emergence at 21°C (%)	Pathogen[a] growth at 28°C (mm)	Seedling[b] emergence at 27°C (%)
Pythium ultimum				
1501 O	38	0	54	9
Rhizoctonia solani				
111 A	19	20	29	38
118 O	18	1	25	14
1118 Pa	13	85	17	69
Colletotrichum gossypii				
611 O	6	89	13	96
612 Mn	6	85	13	94
Fusarium oxysporum				
2018 F	7	96	12	99
F. oxysporum f. *vasinfectum*				
211 Mn	5	91	12	96
F. moniliforme				
212 Mn	6	99	11	92
Aspergillus flavus				
411 O	5	90	11	88
Thielaviopsis basicola				
1313 O	2	100	3	99

[a] Growth in culture in 48 hours.
[b] Emergence counts were taken at the end of 12 days.

Results of this study emphasize the importance of temperature in cotton seedling disease. The data indicate that the optimum temperature for disease is often below the optimum for growth of either host or pathogen. Arndt (3) reported that the optimum temperature for cotton seedling growth is in the vicinity of 30°C. Camp and Walker (7) found the optimum temperature to be nearer 33°C. Optimum growth temperatures for the pathogenic fungi used in the present studies ranged from 24 to 32°C, with the majority growing best at 28°C. In general, disease incidence was greater at 21°C than at higher temperatures, which, although more advantageous to both host and pathogen, apparently gave greater relative advantage to the host than to the pathogen. Leach (11), in studies with preemergence damping-off, showed that disease incidence at different temperatures was determined to a large extent by the relative growth rates of host and pathogen. Cotton, a high-temperature crop, is subjected to an increasingly greater relative disadvantage in comparison with the pathogen when temperatures are progressively lowered.

In these tests the more pathogenic fungi were able

to produce a high incidence of disease over a wider temperature range than the moderately pathogenic species. Some of the most virulent types, such as *R. solani* and *P. ultimum*, were preemergence killers in pure culture in the greenhouse, although in the field these same fungi are usually unable to kill many seedlings before they emerge, doing their greatest damage as postemergence killers. It is possible that future studies with pathogenic fungi in combination with nonvirulent types will show that the competition and antagonism of other microorganisms in the soil are among the important factors in reducing the virulence of these pathogens in nature.

The rate of emergence of cotton seedlings was depressed to some extent by all fungi included in the present experiments. Nonvirulent fungus isolates seemed to affect cottonseed germination adversely. This may account for the fact that cottonseed often does not emerge as well in the field as in laboratory germination tests. In connection with this same phenomenon, two of the most virulent fungi, *C. gossypii* and *T. basicola*, did not reduce seedling emergence appreciably more than the least virulent fungus, yet eventually killed most of the seedlings after emergence. Rate of growth of mycelium appears to be a major factor, but there is some evidence that the mechanism of pathogenicity for the preemergence killing fungi differs somewhat from that of the postemergence killers. Some fungi apparently can attack the seed radicle much more readily than others.

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LITERATURE CITED

1. ARNDT, C. H. 1935. The etiology of damping off of cotton seedlings. (Abstr.) Phytopathology **25**: 968-969.
2. ARNDT, C. H. 1943. Pythium ultimum and the damping off of cotton seedlings. Phytopathology **33**: 607-611.
3. ARNDT, C. H. 1957. Temperature as a factor in the infection of cotton seedlings by ten pathogens. Plant Disease Reptr. Suppl. 246. 84 p.
4. ATKINSON, G. F. 1892. Some diseases of cotton. Alabama Polytech. Inst. Agr. Expt. Sta. Bull. 41. p. 30-39.
5. BLANK, L. M., P. J. LEYENDECKER, JR., AND R. M. NAKAYAMA. 1953. Observations on black root rot symptoms on cotton seedlings at different soil temperatures. Plant Disease Reptr. **37**: 473-476.
6. CAMP, A. F. 1928. Cotton investigations. Florida Univ. Agr. Expt. Stas. Gainesville Ann. Rept. (1928) 38R-39R.
7. CAMP, A. F., AND M. N. WALKER. 1927. Soil temperature studies with cotton. II. The relation of soil temperature to the germination and growth of cotton. Florida Univ. Agr. Expt. Stas. Gainesville Bull. **189**: 17-32.
8. FULTON, N. D., AND KATHARINA BOLLENBACHER. 1958. Pathogenicity of fungi isolated from diseased cotton seedlings in Arkansas. (Abstr.) Phytopathology **48**: 343.
9. GLOVER, T. 1856. Accidents and diseases of the cotton plant. *In* report on agriculture by the commissioner of patents for the year 1855. p. 230-234. U. S. Patent Office Report, Washington. 488 p.
10. KOEHLER, B., AND C. M. WOODWORTH. 1938. Corn-seedling virescence caused by Aspergillus flavus and A. tamarii. Phytopathology **28**: 811-823.
11. LEACH, L. D. 1947. Growth rates of host and pathogen as factors determining the severity of preemergence damping-off. J. Agr. Research **75**: 161-179.
12. LEHMAN, S. G. 1938. Seed infestation with Glomerella and Fusarium in the 1936 cotton crop in North Carolina. Plant Disease Reptr. **22**: 4-6.
13. MARSH, P. B., KATHARINA BOLLENBACHER, J. P. SAN ANTONIO, AND G. V. MEROLA. 1955. Observations on certain fluorescent spots in raw cotton associated with the growth of microorganisms. Textile Research J. **25**: 1007-1016.
14. MILLER, P. R., AND R. WEINDLING. 1939. A survey of cotton seedling diseases in 1939 and the fungi associated with them. Plant Disease Reptr. **23**: 210-214.
15. MILLER, P. R., AND R. WEINDLING. 1940. A survey of cotton seedling diseases in 1940 and the fungi associated with them. Plant Disease Reptr. **24**: 260-263.
16. PRATT, CLARA A. 1926. A disease of Queensland cotton seed. Empire Cotton Growing Review **3**: 103-111. Empire Cotton Growing Corporation, London.
17. RAY, W. W., AND J. H. MCLAUGHLIN. 1942. Isolation and infection tests with seed- and soil-borne cotton pathogens. Phytopathology **32**: 233-238.
18. ROSEN, H. R. 1925. Fusarium vasinfectum and the damping off of cotton seedlings. Phytopathology **15**: 486-488.
19. SCHWEGMANN, J. C. 1953. Temperature-pathogenicity relationships in Rhizoctonia solani on cotton. Plant Disease Reptr. **37**: 178.
20. SHERBAKOFF, C. D. 1940. An occurrence on cotton of black root rot caused by Thielaviopsis basicola. (Abstr.) Phytopathology **30**: 707.
21. SNYDER, W. C., AND H. N. HANSEN. 1940. The species concept in Fusarium. Am. J. Botany **27**: 64-67.
22. WALKER, M. N. 1928. Soil temperature studies with cotton. III. Relation of soil temperature and soil moisture to the soreshin disease of cotton. Florida Univ. Agr. Expt. Stas. Gainesville Bull. **197**: 343-471.
23. WEINDLING, R., P. R. MILLER, AND A. J. ULSTRUP. 1941. Fungi associated with diseases of cotton seedlings and bolls, with special consideration of Glomerella gossypii. Phytopathology **31**: 158-167.
24. WOODROOF, N. C. 1927. A disease of cotton roots produced by Fusarium moniliforme Sheld. Phytopathology **17**: 227-238.

THE DETACHED-LEAF TECHNIQUE FOR TESTING ALFALFA CLONES FOR RESISTANCE TO BLACK STEM [1]

C. H. Ward

SUMMARY

Detached-leaf testing was found to be a reliable method for evaluating resistance in alfalfa to the black stem disease caused by *Phoma herbarum* var. *medicaginis*. Disease records did not differ greatly when the same clones were inoculated in the field or in the greenhouse or when leaves detached from the clones were inoculated in the laboratory. Typical black stem leaf symptoms developed on detached alfalfa leaves within 4–5 days of inoculation. An unsterilized 2.0% sucrose solution was found most satisfactory for culturing alfalfa leaves. Leaves stayed green longer on a 5.0% solution than on a 2.0% solution, but occasional contamination precluded use of the higher concentration. Although detached leaves retained vigor longer in indirect light than in the dark, satisfactory data were obtained from inoculated leaves kept in the dark. Potato-dextrose agar, sterilized alfalfa stems, and potato-dextrose broth are all satisfactory for producing large quantities of the black stem pathogen for inoculations. No differences in morphology, growth habit, or pathogenicity could be distinguished among 4 New York isolates of the pathogen or between a Pennsylvania isolate and the New York isolates. Pathogenicity decreased rapidly when fragmented cultures were kept at 3°C, but intact cultures retained their orginal level of virulence for at least a month when stored at this temperature. Plants inoculated in the greenhouse with spores alone did not become as severely diseased as plants inoculated with a mixture of mycelium and spores. Field inoculations successfully induced epiphytotics, and are highly recommended in situations where there might be, under natural conditions, insufficient infection for accurate disease readings.

Black stem of alfalfa (*Medicago sativa* L.), caused by *Phoma herbarum* West. var. *medicaginis* Fckl. [= *Ascochyta imperfecta* Pk.], can be controlled to some extent by early cutting and by using varieties composed mostly of plants having some resistance (3, 8, 18, 19, 26).

A quick, accurate technique for testing individual plants for resistance to this disease has not been available. Field readings are likely to be unreliable because of extreme seasonal geographic variations in disease occurrence. Greenhouse testing under controlled conditions provides accurate results, but prohibitive amounts of time and space are involved when there are large numbers of plants. Seedlings that are agronomically desirable, but susceptible, may be killed in testing and thus lost from breeding programs. A rapid detached-leaf screening method would be of benefit in developing varieties resistant to black stem, provided results were comparable to those obtainable from greenhouse and field testing programs.

A detached-leaf method for testing alfalfa for black stem resistance was described by Mead (12), but its reliability in comparison with greenhouse methods and field readings has not been determined. The fact that typical black stem symptoms develop on detached alfalfa leaves inoculated with the causal organism (20) suggested that this technique might be of use in screening alfalfa clones for resistance to black stem. Consequently, this possibility was investigated, and experiments were designed to determine the optimum conditions for such tests.

The literature on the use of detached-leaf culture was reviewed in 1946 (29). Its greatest use in plant pathology has been in work with obligate parasites (10, 11, 23, 29). Since 1946, detached-leaf culture has been used for investigations on potato late blight caused by *Phytophthora infestans* (Mont.) d By. (4, 5, 6, 9, 15, 25, 27); these included studies on infection phenomena, testing for resistance, and genetical analyses of resistance. Detached-leaf culture has also been found useful in research on pathogenic race differentiation (7, 22), on sex behavior of rust fungi (16), in screening antibiotics (28), and on the Botrytis disease of gladiolus (17). Testing of tomato and other *Lycopersicon* species for resistance to early blight and Septoria leaf spot has been facilitated by using detached-leaf culture (11).

TESTS WITH DETACHED LEAVES.—*Inocula.*—Three methods of growing inoculum were investigated to determine if a single procedure could produce great numbers of spores and large amounts of mycelium.

First, transfers of the pathogen were made to solidified PDA (potato-dextrose agar)[2] in Petri dishes; these then were incubated at 21–22°C, the reported optimum temperature for growth of *Phoma herbarum* var. *medicaginis* in culture (18). After 21 days, the cultures showed good mycelial growth and heavy pycnidial production, and marginal growth of the colonies had just reached the edges of the Petri dishes.

In the second method, the pathogen was grown on 3- to 5-in. sterilized alfalfa stems in test tubes containing about 10 ml of 2% water agar. The pathogen was transferred to the stems by placing pieces of mycelium at their middle points or by drawing wire loops filled with a spore suspension over their entire lengths. All tubes were incubated at 21–22°C. The fungus grew

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[2] PDA consisted of broth from 200 g potatoes, 20 g dextrose, and 20 g agar diluted with water to make up 1000 ml.

poorly on alfalfa stems infested with pieces of mycelium, but it grew well and sporulated heavily within 10 days on stems infested with a spore suspension.

In the third culturing method, the medium was liquid PDB (potato-dextrose broth)[3] autoclaved 20 minutes at 15 lb. pressure. A disk cut with a sterilized No. 9 cork borer from a fruiting culture of the fungus on PDA was placed in each of 9 Erlenmeyer flasks. Three flasks were kept at 21–22°C in a water-bath shaking machine, 3 were kept at 21–22°C in an incubator, and the remaining 3 were kept in a laboratory cabinet (17–24°C). All cultures were in the dark. Light has been shown to have little or no effect on growth and sporulation of this organism (21). Dry weights of mycelial mats were taken after 21 days. The average dry weight of the mats was 805.4 mg for those in the shaking machine, 648.8 mg for those not shaken, and 637.1 mg for those kept at room temperature.

Although mycelial growth was best in the shake cultures, the cultures grown on PDA in Petri dishes were most convenient and satisfactory for the work reported here. Cultures on PDA in Petri dishes as well as mycelial mats in PDB were suspended in sterilized distilled water and fragmented in a Waring Blendor. On the basis of earlier work (2), fragmented Petri-dish cultures were expected to be satisfactory inocula. Spore suspensions in sterilized distilled water were made from cultures growing on alfalfa stems in test tubes and from cultures growing on PDA in Petri dishes. Pycnidia were broken up by rubbing the surfaces of the cultures with a transfer needle. The spores were taken up in water, and the suspension was filtered through several layers of cheesecloth to remove large particles.

Good infection was obtained on detached leaves with inoculum grown by any of the methods used. In the greenhouse, however, inoculations with spore suspensions resulted in much less infection than inoculations with fragmented cultures containing mycelium and spores. In order that a uniform inoculation procedure could be used in later comparative tests in the laboratory and in the greenhouse, inoculum consisting of fragmented mycelium and spores from Petri-dish cultures was used in all subsequent tests.

The first inoculations were made with inoculum prepared by fragmenting a 21-day-old Petri-dish culture in 100 ml of sterilized distilled water. When this concentration of inoculum was used, however, detached leaves became so diseased that few differences could be distinguished among those from clones known to differ in susceptibility. It seemed possible that clones highly resistant to inoculation under natural conditions might be highly susceptible to such potent inocula and that differences in resistance could be detected only with more dilute inocula. To test this, 7 plants were selected at random from the variety Ranger, and 3 leaves were picked from each plant to serve as replicates for each of 4 treatments, consisting of inoculation with different dilutions of a standard mycelial suspension. The 4 inocula were: a suspension of a 21-day-old culture fragmented in 100 ml of sterilized distilled water, and 2-, 5-, and 10-fold dilutions of this suspension. All leaves inoculated with the first 2 inocula were severely diseased. Leaves from the 7 plants could be placed in distinct susceptibility groups only when the highest dilution was used. With this treatment, similar disease severity data were obtained consistent with each susceptibility group and the lesions could be counted. This concentration, equivalent to 1 culture per 1000 ml of water, was used in all subsequent experiments.

Applicators.—A hand-operated hypodermic syringe equipped with a needle adapted to deliver a measured quantity of mist (13) was tried, but it clogged easily and delivered mist in an unsatisfactory pattern.

A DeVilbiss atomizer attached to a source of air pressure was used. Fragmented mycelium could readily be forced through the atomizer, and the mist pattern was satisfactory. A hand-operated pressure cut-off was placed on the attached air line to allow regulation of the flow of inoculum for delivery of fairly consistent quantities.

Inoculation procedure.—Alfalfa shoots were collected in the greenhouse in the late afternoon (1, 14) and their cut ends immediately placed in test tubes filled with water. As soon as possible, the shoots were taken to the laboratory and washed with distilled water. Leaves were then detached, placed about 6 in. apart on a slightly tilted glass plate, sprayed with inoculum, and transferred with tweezers to sterilized Syracuse watch glasses containing 2.0% sucrose solution. Because of natural curvature, cut ends of petioles were submerged in the solution and leaf blades floated.

It was found that a count of 2 between the time the operator started and stopped the flow of inoculum was sufficient to insure thorough wetting of each leaf. To test the accuracy of this method of controlling the amount of inoculum delivered, the nozzle of the atomizer was placed in each of 40 consecutively numbered test tubes, and a 2-count of delivery was given to each tube. The quantity delivered to each tube varied from 0.78 to 1.06 ml, with a mean of 0.97 ml and a standard deviation of 0.04 ml. Since the differences in the amounts delivered were small, the technique appears satisfactory for the delivery of a fairly uniform inoculum to each leaf.

Leaves from some plants, especially the young leaves, are difficult to wet during inoculation. One drop of Tween 20[4] per 1000 ml of inoculum was found helpful in wetting these leaves, and had no apparent fungitoxic effect.

Effect of sucrose concentration and of light on longevity of inoculated and uninoculated detached alfalfa leaves.—Sucrose, which has been found satisfactory for maintaining detached leaves of leguminous forage plants (29), was used as the substrate. In preliminary tests, a 2.0% solution was chosen on the

[3] PDB consisted of broth from 200 g potatoes and 20 g dextrose diluted with water to make up 1000 ml.

[4] A wetting agent manufactured by Chemicals Dept., Atlas Powder Co., Wilmington, Delaware.

basis of earlier work (20). Contaminated cultures were practically nonexistent, regardless of whether the sucrose solutions were sterilized or unsterilized. Consequently, unsterilized solutions were used routinely.

Preliminary experiments indicated that disease severity data should be taken on inoculated detached leaves 4–5 days after inoculation. Symptoms were clear by the fourth day, but a few leaves showed evidence of chlorophyll breakdown by that time. It was therefore of interest to determine whether sucrose concentration has an effect on the longevity of detached leaves and/or the severity of infection.

Although it had been observed earlier that inoculated detached leaves became as severely infected in the dark as in the light, it seemed reasonable that light might affect the longevity of inoculated and uninoculated detached leaves. Experiments were designed to obtain information on the importance of these factors with various kinds of inoculum.

1) *Materials and methods.*—Two alfalfa plants, one resistant and one susceptible as judged from preliminary tests, were selected from each of the varieties Ranger, Grimm, DuPuits, and Narragansett. A spore suspension (10^6 spores/ml) and a suspension of mycelium and spores (21-day-old PDA culture/1000 ml) were used as inocula. Sucrose solutions of 2.0, 5.0, and 10.0% were prepared by dissolving appropriate amounts of C.P. sucrose in sterilized distilled water.

Detached leaves were collected and inoculated according to procedures described previously. Inoculated leaves were transferred with tweezers to the different sucrose solutions in Syracuse watch glasses. Those to be kept in the light were placed on a laboratory bench, in indirect light since detached leaves in small closed dishes exposed to direct sunlight are likely to be injured or killed by heat (29). The temperature varied from 18° to 23°C. Leaves to receive the dark treatment were placed in a dark chamber maintained at 21–22°C.

A split split-plot design was used. The 4 main plots were varieties of alfalfa; 1 resistant and 1 susceptible plant were used from each variety. The split plots were 3 inoculation treatments: sprayed with water only, inoculated with mycelium-spore suspension, and inoculated with spores alone. The split split-plots in this design were sucrose concentrations of 0, 2.0, 5.0, and 10.0%.

Although disease readings can be made on leaves for several days after the first evidence of yellowing, it is difficult to determine precisely when a leaf has deteriorated to the extent that a satisfactory reading of disease severity cannot be made. To obtain reliable data, therefore, the number of days that each leaf remained completely green was recorded.

2) *Results.*—The mean number of days that leaves of the 4 varieties survived without apparent breakdown was 6.7 for DuPuits, 8.8 for Ranger, 9.8 for Grimm, and 10.6 for Narragansett. This variance was statistically significant at the 5% level (*F* test). Since there was no apparent interaction between varieties and any of the other treatments, data on individual varieties are not given.

In every instance, leaves kept in the light stayed green longer than leaves kept in the dark (Table 1). In the absence of light, longevity of detached leaves increased as the concentration of sucrose was increased to 10%, the highest concentration tested. In the light, however, longevity was generally not as great on 10.0% sucrose as on 5.0%, presumably because photosynthesis in illuminated leaves offset the influence of sugar at the lower concentrations used.

Uninoculated leaves survived longer than did inoculated ones, and those inoculated with mycelium and spores survived slightly longer than those inoculated with spores only.

Since disease data can be taken most advantageously on detached leaves 4-5 days after inoculation, it appears from the mean values in Table 1 that any of the conditions tested would keep leaves alive in good condition for a long enough period. Consideration of the minimum periods of survival, however, would eliminate the use of water. Leaves from some plants survived fewer than 4 days when placed on water, whereas all leaves on sugar solution survived at least 4 days, and in almost all cases survived 5 days or more. It was noticed that contamination was fairly common on 5.0 or 10.0% sucrose, but was rare on 2.0% solutions. Two per cent sucrose solution therefore ap-

TABLE 1.—*Chlorophyll breakdown of detached leaves of 4 alfalfa varieties, both uninoculated and inoculated with Phoma herbarum var. medicaginis, when floated on sucrose solutions of various concentrations in the light and in the dark*

		No. days[a] before breakdown				
	%	Resistant		Susceptible		
Inocula	sucrose	Light	Dark	Light	Dark	Mean
None	0	11.8	7.0	12.0	6.3	9.3
	2	16.3	7.3	11.5	5.0	10.0
	5	15.5	8.8	13.8	8.0	11.5
	10	14.3	8.8	8.3	7.5	9.7
Mean		14.4	7.9	11.4	6.7	10.1
Mycelium-spores	0	10.5	6.2	8.5	5.3	7.6
	2	13.0	6.5	9.8	5.0	8.6
	5	12.0	7.3	12.8	5.8	9.4
	10	11.3	8.5	10.3	6.0	9.0
Mean		11.7	7.1	10.3	5.5	8.7
Spores	0	8.5	5.8	8.5	4.8	6.9
	2	10.8	6.0	7.8	5.0	7.4
	5	12.3	6.3	10.5	6.0	8.8
	10	11.5	8.8	12.0	6.8	9.8
Mean		10.8	6.7	9.7	5.6	8.2
All treatments (means)	0	10.3	6.3	9.7	5.4	7.9
	2	13.3	6.6	9.7	5.0	8.6
	5	13.2	7.4	12.3	6.6	9.9
	10	12.3	8.7	10.2	6.8	9.5
Mean		12.3	7.3	10.5	5.9	9.0

[a] Each figure is the mean of 4 observations. The variance due to sucrose concentration and that due to inoculum were statistically significant at the 1% level (*F* test). Variance due to reaction type (resistant vs. susceptible) was significant at the 5% level. The interactions between any 2 of these sources of variance, and those among all 3, were not statistically significant.

peared to be the most satisfactory for work with the black stem pathogen on alfalfa leaves.

Pathogenicity of 2 different isolates of P. herbarium var. medicaginis.—Different isolates of *P. herbarum* var. *medicaginis* collected in New York were indistinguishable as to morphology, growth habits, and pathogenicity; there was no evidence of physiologic specialization.

An isolate of the fungus from Pennsylvania[5] could not be distinguished from New York isolates on the basis of morphology. No differences in pathogenicity of the Pennsylvania isolate and a representative New York isolate could be distinguished, as judged from infection experiments using matched leaves selected from 5 plants of each of the varieties Ranger, Grimm, Narragansett, and DuPuits. A New York isolate was used in subsequent screening experiments.

Effect of prolonged storage at low temperature on the pathogenicity of P. herbarum var. medicaginis.—A detached-leaf testing technique would be particularly useful in a breeding program in connection with evaluation of disease reactions of greenhouse-grown progenies from controlled crosses before these progenies are planted in the field. Most of such testing would be done over a 3- to 4-week period, which would necessitate large quantities of inoculum of uniform virulence. If cultures could be grown under controlled conditions for a certain period and then stored in the cold without loss or change in pathogenicity, testing of detached leaves would be greatly facilitated. Peterson and Melchers (18) reported that *P. herbarum* var. *medicaginis* grows at 9°C and that spores remained viable for 6 months when stored at 4.4°C (40°F). In the present work this organism did not grow at 3°C, and preliminary tests indicated that cultures stored at 3°C retained full infectivity for 1 week. Further experiments were designed to test the effect of prolonged storage at low temperature on the pathogenicity of the fungus.

1) *Materials and methods.*—Cultures started by transfers to the center of solidified PDA in Petri dishes grow uniformly over the entire surface of the medium and produce pycnidia abundantly in 21 days at 22°C. Cultures of this age were used routinely because most spores were viable and because the cultures were so uniform in growth that dilutions of 1 Petri-dish culture could be used as a measure of inoculum concentration.

Twenty-four cultures were started on PDA in Petri dishes and incubated 19 days at 22°C. Then 12 cultures were placed at 3°C. Each of the other 12 was fragmented with and suspended in 100 ml of sterilized distilled water in a Waring Blendor that had been disinfested by rinsing in 70% ethanol. After fragmentation, each suspension was poured into a sterilized 250-ml Erlenmeyer flask, which was then plugged with sterilized cotton and kept at 3°C.

Before stored cultures were used in pathogenicity tests, they were removed from storage and kept at 22°C for 2 days, making a total of 21 days of active growth. Cultures used for pathogenicity comparisons with stored cultures had been grown 21 days at 22°C on the day they were used. For each test in which stored inoculum was to be compared with fresh inoculum, matched leaves for the inoculation were detached from 4 plants of each of 4 varieties.

The inoculated leaves were floated on 2.0% sucrose solution in Syracuse watch glasses, which were stacked randomly and kept in the dark at 22°C. Pathogenicity tests were made on the fragmented cultures after 2 and 4 weeks of storage. Tests were made on cultures stored in Petri dishes after 2, 4, 8, and 12 weeks of storage. The number of lesions that developed on each leaf 4 days after inoculation was recorded.

2) *Results.*—Fragmented cultures stored for as little as 2 weeks were less pathogenic than were fresh ones; the differences were statistically significant at the 5% level. In these and all subsequent tests, there were highly significant differences among plants in disease reaction; these were expected on the basis of previous tests, and substantiate the fact that plants within varieties vary in susceptibility to black stem.

No statistically significant differences in pathogenicity were obtained between fresh cultures and cultures stored in Petri dishes for 2 or 4 weeks. Cultures stored for either 8 or 12 weeks, however, were significantly more pathogenic than fresh cultures. This was unexpected, but it may be attributed to maturation of spores and to additional spore formation during the cold treatment. Conidia were observed to be exuding more heavily from pycnidia in cultures removed from storage at the end of 12 weeks than from pycnidia in fresh cultures. These results indicate that fragmented stored cultures cannot be used as a source of uniform inoculum, but that cultures stored in Petri dishes for no longer than 4 weeks at 3°C should be a reliable source of inoculum. Cultures stored in Petri dishes for more than 4 weeks should not be used for comparative studies at different times, but would be suitable for field inoculations, or for comparative studies made at the same time.

COMPARISON OF BLACK STEM REACTION OF ALFALFA CLONES IN FIELD, GREENHOUSE, AND LABORATORY TESTS.—Because alfalfa is naturally cross-pollinated and because plants within varieties are highly heterozygous, varieties and plants within varieties react differently to the diseases that attack them. This is especially true with black stem, for resistance is inherited through several factors (24). Individuals that possess a high level of resistance to black stem have been selected from susceptible commercial varieties (10).

For comparative tests with the 3 methods, it was thought desirable to use random samples of plants drawn from commercially grown varieties rather than to use clones known to be resistant. If the gradations in disease reaction were the same in all of the 3 methods of testing, then one could be reasonably sure that the 3 testing methods are comparable and that observed differences in disease reaction are true ones.

Materials and methods.—Certified seed of 4 commercially grown alfalfa varieties, Ranger, DuPuits, Grimm, and Narragansett, were planted in clay pots

[5] Obtained from J. H. Graham, U. S. Regional Pasture Laboratory, University Park, Pennsylvania.

in the greenhouse. Thirty-five pots of each variety were planted, and seedlings were thinned to 1 per pot after 3 weeks of growth. Each plant was vegetatively propagated by stem cuttings when 3 months old. Cuttings were taken near the centers of the stems and rooted in moist builders' sand. After rooting, the cuttings were transplanted to clay pots and allowed to grow. In June, 1956, some of these cuttings were used for the planting of a replicated spaced-plant nursery adjacent to the Cornell alfalfa breeding plots. A randomized block design with 4 replicates was used. Replications included 18 plants from each of the 4 varieties. The plot thus contained 288 plants. Guard rows consisting of miscellaneous alfalfa plants were planted around the entire plot.

Natural infection was so slight during the first summer that satisfactory disease ratings could not be made. Plants that did not survive the winter were replaced by greenhouse-grown clones early the following spring. The incidence of infection during the spring of 1957 was small, even though cool, wet weather prevailed. In June, the plants were inoculated heavily with a mycelium-spore suspension of a representative New York isolate of *P. herbarum* var. *medicaginis*. Inoculum was applied at dusk with a hand-operated pressure-tank sprayer. The guard row was not inoculated. Two other inoculations were made at weekly intervals. Dew was heavy on the nights the plants were inoculated, and excellent infection developed on the inoculated plants; uninoculated plants in the guard rows, however, remained practically free from disease for at least 2 weeks after the last inoculation. Disease severity readings were made 10 days after the last inoculation. Readings were on the basis of a scale of 0–10, from no infection to death of plants. Leaf spotting and stem lesions were used as criteria for rating disease severity.

Plants of the same clones used in the field, obtained from cuttings, were inoculated in the greenhouse. Moisture-chamber facilities would accommodate only about 100 plants in 4-in. pots; therefore, only 1 representative of each plant was inoculated at any 1 time. After disease severity ratings had been made, the plants were cut back to the crown and allowed to regrow. Regrowth was disease-free, and the same plants were used successfully for second, third, and fourth inoculations.

One week before each inoculation, the plants were pruned to about the same size and shape. Inoculum, consisting of a mycelium-spore suspension, was applied with a DeVilbiss atomizer with forced air. Plants were sprayed with inoculum until run-off, and then placed in a chamber maintained at 68°F and about 100% relative humidity. Three days after inoculation, the plants were removed from the chamber and placed on a greenhouse bench. Disease severity was scored 7 days after inoculation; the scale was that used in the field.

Two experiments were carried out in the laboratory, on different dates, with detached leaves from the parent clones of the plants tested in the field and greenhouse. Detached leaves were inoculated with a mycelium-spore suspension of the pathogen, floated on 2.0% sucrose solution in Syracuse watch glasses, and placed in indirect light. Procedures followed for collecting and inoculating detached leaves and for growing and preparing inoculum were those described in earlier sections.

In 1 experiment, the 4 leaves from each plant, used as replicates, were of different age groups. The youngest leaves (replicate 1) were detached from near the tops of the plants, whereas the oldest leaves (replicate 4) were taken from the lower portions of the plants. Four days after inoculation, data were taken on the average number of spots per leaflet.

In another experiment, the 4 leaves for replicates were closely matched for size and age, and disease severity readings were made 5 days after inoculation.

Results.—Data from the first detached-leaf experiment indicate that differences in disease reaction were highly significant among varieties and also among plants within the varieties tested. Young and old leaves from the same plant were found equally susceptible. This is in agreement with the report by Peterson and Melchers (18). Four replicates were found to be sufficient to sample the inherent variability in disease reaction among leaves of a single plant.

In the first experiment, data were recorded as number of lesions in an attempt to reduce error in laboratory readings by putting them on an absolute basis. These ratings were converted to ratings on a scale of 0–10 and compared with greenhouse and field ratings. Because ratings based only on numbers of lesions were not strictly comparable, size and number of lesions were used as criteria in rating diseased leaves on a 0–10 scale in all other experiments.

TABLE 2.—*Disease ratings of identical clonal lines of 4 alfalfa varieties inoculated with Phoma herbarum var. medicaginis in the field (whole plants), in the greenhouse (whole plants), and in the laboratory (detached leaves)*

	Disease ratings [a]					
	Field		Greenhouse		Laboratory	
Varieties	Range	Mean	Range	Mean	Range	Mean
Narragansett	3.50–6.25	4.84	3.75–6.75	5.38	3.75–7.00	5.43
Ranger	5.25–7.25	5.90	4.25–7.00	5.52	4.50–7.50	5.79
Grimm	5.00–7.25	6.03	5.00–7.00	6.06	5.25–7.75	6.40
DuPuits	6.00–7.75	6.76	5.00–7.75	6.62	5.50–7.75	6.56

[a] Each figure is the mean or range for 18 observations. Ratings based on a scale of 0–10, with 0 indicating no infection and 10 indicating death of leaves.

TABLE 3.—*Correlation coefficients for comparisons of disease severity ratings of 4 alfalfa varieties inoculated with Phoma herbarum var. medicaginis in the field (whole plants), in the greenhouse (whole plants), and in the laboratory (detached leaves)*

Varieties	Comparisons		
	Field-greenhouse	Field-laboratory	Greenhouse-laboratory
Narragansett	0.713**	0.624**	0.766**
Ranger	0.556*	0.510*	0.497*
Grimm	0.410	0.411	0.534*
DuPuits	0.581*	0.595**	0.757**

*Denotes significance where P = 0.05.
**Denotes significance where P = 0.01.

Disease ratings of detached alfalfa leaves did not vary greatly from those obtained by field and greenhouse methods (Table 2). Ratings taken on detached leaves in the laboratory were usually higher than ratings taken in the field and greenhouse, although the highest mean readings for Ranger and DuPuits were obtained in the field. Differences were evident in the disease reactions of the 4 varieties tested, and these differences occurred irrespective of the method of testing. DuPuits consistently gave the highest disease readings, whereas Narrangansett was most resistant and gave the lowest readings. Since only a small sample was taken of each variety, it is not intended to generalize on varietal susceptibility to black stem, but these data serve as an indication that there are varietal differences. The ranges of disease severity ratings were greatest when detached leaves were used. This indicates that perhaps smaller differences in disease reaction can be distinguished by testing detached leaves than by testing whole plants.

Reasonably good correlations were obtained for the comparisons of the laboratory, greenhouse, and field disease-severity readings (Table 3). Failure to obtain significant correlations for the laboratory-field and greenhouse-field readings for the variety Grimm can be attributed to several aberrant observations in the field data. Although all plants tested in the field were uniformly inoculated, variation in the amounts of inoculum received by different plants or variations in the environment within the plot were doubtless greater than such variation in the greenhouse or laboratory, and could have caused the aberrant results. Although these correlation coefficients are not high, most are statistically significant, and they serve to show that detached-leaf testing can be used as a reliable method of evaluating disease reaction of individual plants to black stem. This method is certainly as reliable as either of the other 2. It is also evident that greenhouse and field testing can be used profitably for determining black stem disease reactions if proper precautions are taken. One important precaution is controlled inoculation in the field.

DISCUSSION.—The advantages and disadvantages of using detached-leaf testing for disease reaction and for other experimental work involving pathogens have been described (29). Economy of time and money involved in screening large populations of alfalfa plants by using detached-leaf testing is believed to be a significant implication of the work reported here.

Detached-leaf culture offers a quick, accurate method of testing alfalfa plants for resistance to black stem. It could be used extensively in testing programs or could be reserved for specialized small-scale work where controlled conditions are necessary. It could be used also for checking greenhouse and field results. In this work, detached leaves were used for screening only 1 forage crop for resistance to 1 disease; with a little effort, however, the technique could probably be adapted for the screening of many forage legumes for resistance to other diseases.

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LITERATURE CITED

1. CURTIS, O. F. 1944. The food content of forage crops as influenced by time of day at which they are cut. J. Am. Soc. Agron. **36**: 401-416.
2. GRANDFIELD, C. O., E. D. HANSING, AND H. L. HACKEROTT. 1948. Losses incurred in asexual propagation of alfalfa clones. J. Am. Soc. Agron. **40**: 804-808.
3. HELGASON, S. B. 1951. Effect of disease on alfalfa lines and hybrid combinations in relation to seed production. *In* Reports of Prairie Regional Committee. Natl. Research Coun. Review, Canada, p. 216.
4. HOWATT, J. L., AND P. N. GRAINGER. 1955. Some new findings concerning Phytophthora infestans (Mont.) de By. Am. Potato J. **32**: 180-188.
5. HOWATT, J. L., AND W. A. HODGSON. 1954. Testing for late blight resistance in the potato in Canada. Am. Potato J. **31**: 129-140.
6. HOYMAN, W. G. 1957. Comparative reactions of attached and detached leaves to Phytophthora infestans. (Abstr.) Phytopathology **47**: 17.
7. JENKINS, W. R. 1954. Pathogenic races of Diplocarpon rosae, the rose blackspot fungus. (Abstr.) Phytopathology **44**: 389.
8. JOHNSON, E. M., AND W. D. VALLEAU. 1933. Blackstem of alfalfa, red clover and sweet clover. Kentucky Agr. Expt. Sta., Univ. Kentucky Bull. **339**: 55-82.
9. KEAY, M. A. 1954. Methods for studying the susceptibility of potato foliage to Phytophthora infestans. Plant Pathol. **3**: 131-132.
10. KOEPPER, J. M. 1942. Relative resistance of alfalfa species and varieties to rust caused by Uromyces striatus. Phytopathology **32**: 1048-1057.
11. LOCKE, S. B. 1948. A method for measuring resistance to defoliation diseases in tomato and other Lycopersicon species. Phytopathology **38**: 937-942.
12. MEAD, H. W. 1954. Detached leaf method. *In* Report of the Committee on Screening Alfalfa Breeding Stocks, by R. G. Henderson. Report of the Fourteenth Alfalfa Improvement Conference, p. 51.
13. MILLER, P. M. 1956. Use of hypodermic syringe and needle as an atomizer. Plant Disease Reptr. **40**: 755.
14. MILLER, P. R. 1932. Pathogenicity of three red-cedar rusts that occur on apple. Phytopathology **22**: 723-740.
15. MÜLLER, K. O., J. C. CULLEN, AND M. KOSTROWICKA. 1955. Testing "true resistance" of the potato to blight, Phytophthora infestans. J. Natl. Inst. Agr. Botany **7**: 341-354.
16. PAYAK, M. M. 1955. A modified Petri dish method for

rust infection of excised leaves. Experientia **11:** 239-241.

17. Peirs, J. W. L. 1949. The Botrytis disease of Gladiolus with special reference to the causal organism. Brit. Mycol. Soc. Trans. **32:** 291-304.
18. Peterson, M. L., and L. E. Melchers. 1942. Studies on black stem of alfalfa caused by Ascochyta imperfecta. Phytopathology **32:** 590-597.
19. Reitz, L. P., C. O. Grandfield, M. L. Peterson, G. V. Godding, M. A. Arneson, and E. D. Hansing. 1948. Reaction of alfalfa varieties, selections, and hybrids to Ascochyta imperfecta. J. Agr. Research **76:** 307-323.
20. Roberts, D. A. 1956. Use of a detached-leaf technique for testing leguminous forage plants for resistance to foliage diseases. (Abstr.) Phytopathology **46:** 24.
21. Schenck, N. C., and J. W. Gerdemann. 1956. Taxonomy, pathogenicity, and host-parasite relations of Phoma trifolii and Phoma herbarum var. medicaginis. Phytopathology **46:** 194-200.
22. Sherwood, R. T. 1957. Physiologic races of the red clover leaf rust fungus. Phytopathology **47:** 495-498.
23. Silverman, W. B. 1957. The development of Puccinia graminis var. tritici on detached leaves of wheat as influenced by nutrients and metabolite antagonists. (Abstr.) Phytopathology **47:** 31-32.
24. Stevenson, F. F., and H. A. Jones. 1953. Some sources of resistance in crop plants. *In* Plant Diseases. Yearbook Agr., 1953, U. S. Dept. Agr. p. 192-216.
25. Takase, N., and M. Takakuwa. 1956. Studies on the resistance to late blight in potatoes. II. Field infection of interspecific hybrid potatoes with newly appeared strain of Phytophthora infestans. Japan J. Breeding **6:** 1-4.
26. Toovey, F. W., J. M. Waterston, and F. T. Brooks. 1936. Observations on the black-stem disease of lucerne in Britain. Ann. Appl. Biol. **23:** 705-717.
27. Toxopeus, H. J. 1954. Leaf testing as a method of genetical analysis of immunity from Phytophthora infestans in potatoes. Euphytica **3:** 233-240.
28. Tsao, P. H., C. Leben, and G. W. Keitt. 1956. Detached leaves for screening antibiotics. (Abstr.) Phytopathology **46:** 29.
29. Yarwood, C. E. 1946. Detached leaf culture. Botan. Rev. **12:** 1-56.